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# THE EFFECTS OF SULPHUR DIOXIDE ON SELECTED HEPATICS

(TITLE)

BY

Steven L. Gatchel

**THESIS**

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

Master of Science

IN THE GRADUATE SCHOOL, EASTERN ILLINOIS UNIVERSITY
CHARLESTON, ILLINOIS

1978
YEAR

I HEREBY RECOMMEND THIS THESIS BE ACCEPTED AS FULFILLING
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# THE EFFECTS OF SULPHUR DIOXIDE ON SELECTED HEPATICS

BY

STEVEN L. GATCHEL
B.S. in Botany, Eastern Illinois University

ABSTRACT OF A THESIS
Submitted in partial fulfillment of the requirements
of the degree of Master of Science in Botany at the Graduate School
of Eastern Illinois University

Charleston, Illinois
1978

Extensive studies have been reported on the air pollutant sulphur dioxide ($SO_2$), and its effects on vascular and nonvascular plants. It has been shown to interrupt normal physiology, metabolism, reproduction, and alter the plant"s morphology.

Of the cryptogams, lichens have been extensively used as biological indicators of air pollution. More recently, bryophytes have been shown to be as sensitive to contaminates as lichens, and may exhibit responses to the pollutant similar to those of vascular and nonvascular plants. The threshold sensitivity of these cryptogams is about 0.5 ppm over a 12 hour period. However no study to date has investigated the utilization of hepatics as possible pollution indicators.

The intent of this research was to investigate the general responses of selected liverworts to sulphur dioxide. The liverworts used were: Blasia pusilla L., Lophocolea heterophylla (Schrad.) Dum., Scapania nemorosa (L.) Dum. and Jamesoniella autumnalis (D.C.) Steph. The thalli were fumigated under varying $SO_2$ concentrations in an ecological chamber for 8 hours. All thalli exhibited a marked discoloration (chlorosis) which varied directly with the $SO_2$ concentration. Chlorophyll analysis showed a 34-37% decrease in total chlorophyll content at 0.4 ppm $SO_2$ concentration. Numerical data concerning Blasia reflects a conflict with chlorophyll extraction procedures of a plant and an algae. The $SO_2$ caused degradation in chlorophyll$_a$ whether plant or algal, which did account for the loss in total chlorophyll.

These liverworts exhibit a typical response to $SO_2$ and have a threshold equal to, or slightly less than, other cryptogams.

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## Acknowledgements

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## Introduction

The U.S. Department of Health, Education, and Welfare defines air pollution as:

> the presence in the atmosphere of one or more contaminants of such quantities and duration as may be injurious to human, plant, or animal life or property or which unreasonably interferes with comfortable enjoyment of life, property, or conduct of business.

Simply, air pollution is the contamination of the atmosphere, an unfavorable alteration of the components of air. This may be due to either an abnormally high concentration of the normally occurring gases in pure air, or the addition of unwanted airborne matter from natural or man-made sources. Natural pollutants may be pollen, fungi, bacteria, spores, volcanic eruption material, gas seepages, bacterial decay products, etc. These pollutants enter the atmosphere through natural processes, i.e., fire, wind, hot springs, volcanic fissures and eruptions.

Man-made pollution began with the first fires used by the caveman to cook and heat his cave. Early major sources of air pollution were industries concerned with metallurgy, ceramics and animal-product preservation. The forging of copper, gold, and the baking of clay have been recorded before 4000 B.C. Shortly before 1000 B.C. leather tanneries and iron forges were polluting the atmosphere. Coke was the principal form of coal used prior to 1000 B.C., although coal was mined extensively. As early as 500 B.C., crude oil was burned in Persian shrines. Horace noted in his writings the adverse effects of the blackening smoke upon the temples of Rome. In Medieval times, British kings decreed the fouling of London air by smoke a crime, punishable by hanging. In the Middle

Ages, the term "miasmas" described the presence of poisonous airs. The Italian expression for bad air, mala aria, was the basis for the initial concept of malarial infestation from swamp odors. By the beginning of the 14th century, the effects of coal usage upon the air became apparent; dark smoke, unpleasant odors, and the blackening of buildings and monuments.

With the Industrial Revolution, and a subsequent increase in fossil fuel consumption, air pollution levels increased. As a result, major disasters attributed to air pollution began to appear. London had the first recorded major pollution disaster in February, 1880, and the most tragic disaster on record, in December, 1952. Similar disasters occurred in January, 1956; December, 1957; and December, 1962. Other early air pollution disasters occurred in the Meuse Valley, Belgium (Dec., 1930); Donora, Penn. (Oct., 1948); New York (Nov., 1953, Jan., 1963; and Nov., 1966); Cincinnati (Aug., 1968); New Orleans (Oct., 1953); Yokohama, Japan (1956); and in the nonindustrial center of Poca Rica, Mexico (Nov., 1950).

## $SO_2$ Gas and Its Biogeochemical Reactions

Sulphur dioxide ($SO_2$) is a colorless, nonflammable gas, very soluble in water (22% by weight at $0^{\circ}C$). It produces an acrid taste at concentrations of 0.3-1.0 ppm, and a pungent, irritating odor at levels of 3.0 ppm or greater. It is directly produced in volcanic fumes, during biological decay by anaerobic bacteria, and during the combustion of sulphur containing fuels.

Sulphur is a necessary element in the general metabolism of plants, as a major component of amino acids, proteins, and some vitamins. There are specific macroscopic symptoms for sulphur deficient plants, those having concentrations below 0.25 mg S/g dry wt. The sulphur requirement is fulfilled by the uptake of sulphate ions through the roots and, in part, by the direct uptake of atmospheric $SO_2$ or aqueous sulphite ions. Low atmospheric concentrations of $SO_2$ can be utilized by the plant, but concentrations above its biochemical threshold level interfere with basic cellular processes such as photosynthesis, respiration, and metabolism.

### Sulphur Cycle

Figure 1 is a simplified representation of some of the extremely complex reactions involving the various forms of sulphur. No attempt has been made to equate produced and consumed sulphur, as data is difficult to accurately obtain and correlate. The reactions may not always occur in the sequences illustrated, due to prevailing environmental factors such as wind, humidity, particulate matter, aerosols, ultraviolet and visible sunlight, etc. This figure is intended to illustrate the cycling

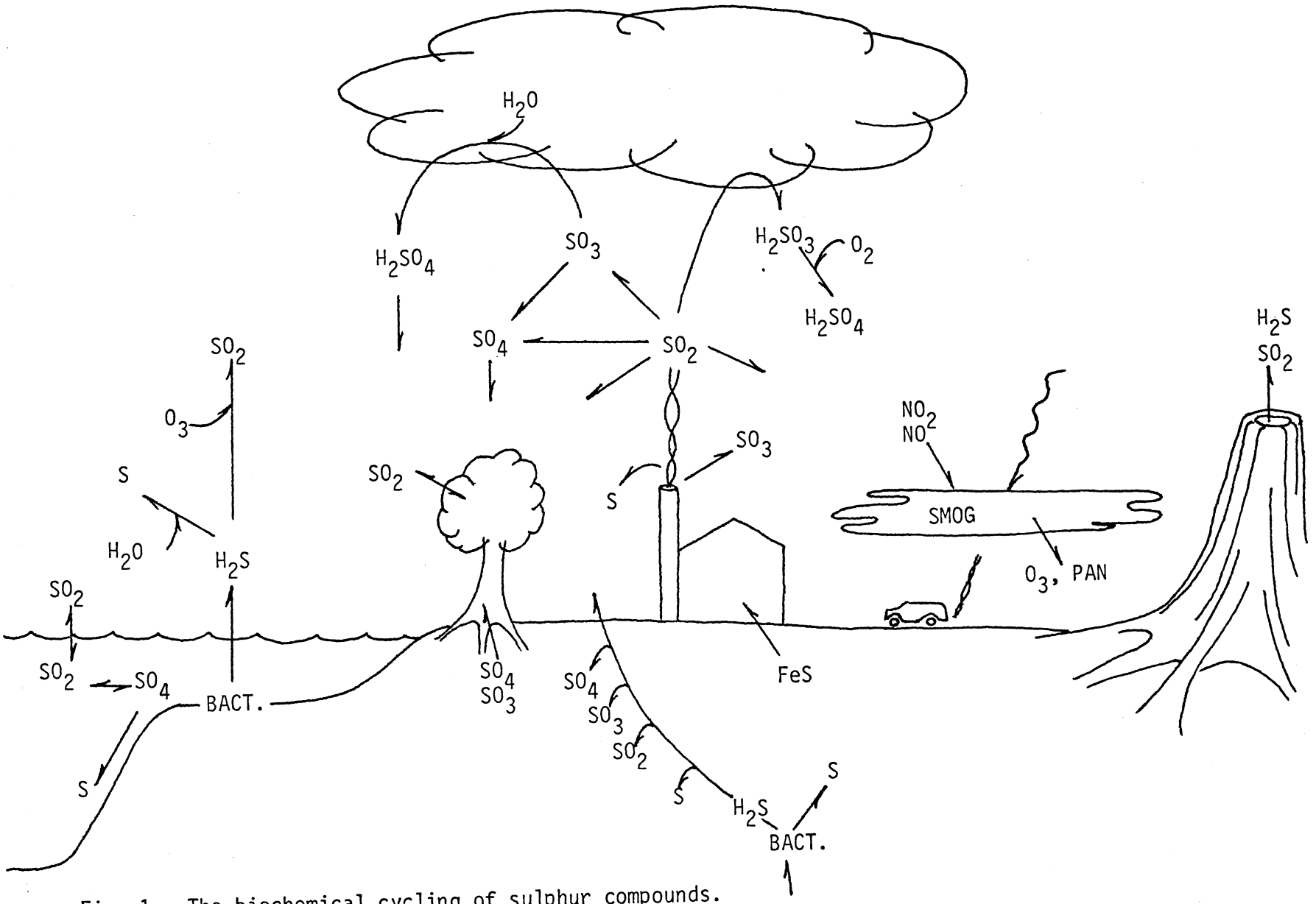



Fig. 1. The biochemical cycling of sulphur compounds.

of the various forms of sulphur with regard to their sources, sinks, and major chemical reactions. Some of the known reactions involving the conversions of one form of sulphur compound to another are listed in Table 1. The majority of the presented information has been obtained from studies by Brosset (1973) and by Kellogg et al (1972), although numerous other publications are also available.

Sulphur enters the atmosphere as a gas, as particulate matter, or as a mist. It enters the soil through the decomposition of organic manures, decomposition of native rock and dissolved in rainwater. Sulphur may be found in all three physical states. As a gas, it is primarily $SO_2$ and $H_2S$. There are transient gaseous states of $HS^-$, $SO^-$, $HSO_3^-$, $SO_3^=$, and $SO_4^=$, but these are readily removed by contact with any surface. Of lesser importance are the various forms of carbon and sulphur compounds called mercaptans (CS, $(CH_3)_2S$, COS, etc.). As a liquid, sulphur is usually found as $H_2SO_4$, $H_2SO_3$, and $SO_2(aq)$. These may be in liquid particle form, or as a film on solid particles. Sulphur as a solid may take on many forms. Sulphates present as fine particles (less than 1 $\mu$) form aerosols, the primary cause of reduced atmospheric visibility. Other incorporations of sulphur are organosulphur compounds, sulphites of transition metals, sulphide ores or compounds such as $(NH_4)_2SO_4$, $(NH_4)_3(HSO_4)_2$, $NH_4HSO_4$, $CaSO_4$, $MgSO_4$, $NaSO_4$, etc. In coal, sulphur occurs in three forms: (1) as part of the complex organic compounds of coal, (2) as sulphate compounds, and (3) as pyrites, fine particles of iron sulphide ($FeS_2$).

It is difficult to determine the components of unpolluted air and their respective proportions. The following Table lists some of the components and their concentrations.

Table 1. Chemical reactions of $SO_2$ and its products.

Source

1. $FeS_2 + O_2 \longrightarrow FeSO_4 + SO_2$
2. $CuS + O_2 \longrightarrow CuO + SO_2$
3. $S + O_2 \longrightarrow SO_2$
4. $S + H_2O + O_2 \longrightarrow H_2SO_4$
5. $H_2S + CO_2 \longrightarrow CH_2O + S + H_2O$
6. $H_2S + SO_2 \longrightarrow H_2O + S$
7. $2C + MeSO_4 + H_2O \longrightarrow MeCO_3 + CO_2 + H_2S$

   when Me = metal, C = organic substrate
8. $2CO_2 + H_2S + H_2O \longrightarrow 2(CH_2O) + H_2O + 2S$
9. $SO_2 + O_2 \longrightarrow SO_3$

Sink

10. $SO_2 + H_2O \longrightarrow H_2SO_3$
11. $H_2SO_3 \longrightarrow H^+ + HSO_3^-$ (pK = 1.76)

    $HSO_3^- \longrightarrow H^+ + SO_3^=$ (pK = 7.20)
12. $SO_2 \xrightarrow{uv} {}^*SO_2 + O_3 \longrightarrow SO_3^=$
13. $SO_2 \xrightarrow{oxd} SO_4^=$
14. $SO_3 + H_2O \longrightarrow H_2SO_4$ or a $SO_4^=$ salt
15. $SO_4^= \xrightarrow{red} SO_2$, S, or $H_2S$
16. $O_2$ + disolved $SO_2 + H_2S \longrightarrow SO_4^=$
17. $H_2S + O \longrightarrow OH^- + HS^-$
18. $H_2S + O_3 \longrightarrow SO_2 + H_2O$
19. $H_2SO_3 + H_2O \longrightarrow H_2SO_4$
20. $H_2SO_3 \xrightarrow{oxd} H_2SO_4$
21. $H_2SO_3 + O_3 \longrightarrow H_2SO_4$
22. $CaSO_4 + H_2 \longrightarrow H_2S + Ca(OH)_2 + H_2O$
23. $H_2SO_4$ + metal oxide $\longrightarrow$ metal $SO_4$

Table 2. Composition of Clean, Dry Air Near Sea Level: in ppm

| | | | |
|---|---|---|---|
| Nitrogen | 780,900 | Methane | 1.5 |
| Oxygen | 209,400 | Hydrogen | 0.5 |
| Argon | 9,300 | Carbon Monoxide | 0.1 |
| Carbon Dioxide | 318 | Ozone | 0.02 |
| Neon | 18 | Nitrogen Dioxide | 0.001 |
| Helium | 5.2 | Sulphur Dioxide | 0.0002 |

Atmospheric Pollution, W. Bach, 1972, McGraw-Hill, Inc., 144 pg.

The various states and chemical forms of sulphur in a polluted atmosphere interact with one another, and with the normally occurring gases and particulates. It is also difficult to determine the residence time of any particular sulphur form in the ecosystem because of the miriad of factors which prevail at any given time. Some of these factors are concerned with the emission of the pollutant (site, height of introduction, physical and chemical form), others depend on the atmosphere (prevailing winds, clouds, temperature, precipitation, light, air-borne particles). Studies indicate that $SO_2$ may be present up to 43 days after emission. Under proper conditions, 30-60 minutes after emission $SO_2$ will reach a state in which it is readily available for washing out. Sulphuric acid ($H_2SO_4$) can be neutralized by ammonia and/or calcium sulphate. Gravity and precipitation can remove $H_2SO_4$ and $SO_4^{=}$. Some residence times for forms of sulphur are: $H_2S$ (1-1.7 days); $H_2SO_4$ (2.4-14 days, depending on the altitude); $SO_4^{=}$ as an aerosol (1-2 years).

With reference to Fig. 1 and Table 1, there are two sources of sulphur dioxide, natural and man-made. Man's pollution of the atmosphere with $SO_2$ is primarily through the combustion of sulphur containing fossil fuels (Rx 1). About 95% of the combustion-produced sulphur compounds are $SO_2$. During combustion, 2-3% of the produced $SO_2$ is oxidized in the exhaust stack. This form, when released, reacts with atmospheric water

vapor to form $H_2SO_4$ (Rx 14), about 5M in strength. This acid is normally dissipated in the immediate area. The remaining $SO_2$, as well as minute amounts of elemental sulphur and $SO_3$, are emitted into the atmosphere. The smelting of sulphide ores of copper, lead, and zinc presents a major point source of $SO_2$ pollution (Rx 2). Increases in the population and in the use of high sulphur-content fuels make these sources a continuing problem.

There are two natural sources of sulphurous compounds, volcanoes and bacteria. Volcanoes and fumaroles emit $SO_2$ and $H_2S$, as well as small amounts of $SO_3^=$, sulphates, and elemental sulphur. The hot eruption clouds containing reduced sulphur compounds are oxidized by atmospheric oxygen, producing $SO_3^=$ which quickly reacts with water vapor, forming $H_2SO_4$ droplets (Rx 9, 14).

Bacterial organisms play an important role in the cycling of sulphur. Prior to man's contribution, bacterial production of $H_2S$ was the primary return path of biologically incorporated sulphur compounds to the atmosphere.

Anaerobic bacteria found in soil, water, and marshy areas, can reduce sulphates to S and $H_2S$. Under anaerobic conditions, $SO_x$ is the source of $O_2$ for the oxidation of organic matter. In the genus Desulfovibrio, sulphate is the terminal electron acceptor in anaerobic respiration, oxidizing available organic matter and producing large amounts of sulphides (Rx 7). Bacterial $H_2S$ may be: (1) oxidized as it filters upward through the soil, depositing sulphides, sulphates, sulphites, and elemental sulphur in the soil, (2) released as $H_2S$ gas to the atmosphere, causing an odor similar to rotten eggs, and/or (3) oxidized to elemental sulphur via photosynthetic sulphur bacteria.

Photosynthetic sulphur bacteria belong to the order Pseudomonales, specifically the Thirohodaceae (purple sulphur) and Chlorobacteriaceae (green sulphur) families. Both families are anaerobic facultative autotrophs, using $CO_2$ as a carbon source (Rx 8) and involving bacteriochlorophyll and carotenoids. Other facultative autotrophic bacteria belong in the families Achromatiaceae, Beggitoaceae, and Thiobacteriaceae.

Sulphur bacteria are chemosynthetic, obtaining energy by the oxidation of inorganic materials for the synthesis of organic compounds. These compounds are then oxidized internally yielding energy to the cell and releasing $CO_2$. Thiobacillus species can oxidize all forms of sulphur into sulphates (Rx 4). These sulphates may be assimilated by plants and incorporated into organic compounds, or may be reduced to provide nutritional sulphur for many bacteria and soil organisms.

Plants utilize several forms of sulphur found in both the soil and the atmosphere. Nutrient supplies of $SO_3^-$ and $SO_4^=$ are found in the soil, dissolved in water.

Depending on a number of factors, an uncertain amount of the released $H_2S$ can be oxidized to $SO_2$. Hydrogen sulphide may also undergo oxidative reactions with forms of oxygen over a period of hours (Rx 17, 18). The reaction of $H_2S$ and $O_3$ (Rx 18) proceeds very slowly except when aerosols are present to provide reactive surfaces.

The oceans are another source of sulphur compounds, as normal sea water contains about 2.65 mg of $SO_4$ per gram of water. Sea salt formed during the breaking of saltwater bubbles is an important source of atmospheric sulphate over oceans. Hydrogen sulphide is also contributed from ocean sources, but this is generally restricted to tidal flats. Any $H_2S$ liberated from the sea bottom is rapidly oxidized by the dissolved

oxygen in the water, accounting for the inability of present-day techniques to measure the minute concentrations of $H_2S$ in sea water (Rx 16). Another possible source-mechanism of the ocean is the equilibrium vapor pressure of the soluble $SO_2$ in water, with that of the partial pressure in the air immediately above it. The pH of ocean water is about 8.1, and this will tend to increase the rate of oxidation of $SO_2$ to sulphates. Whether this oxidation rate is fast enough to account for the equilibrium and measurable $SO_4^=$ remains to be tested.

All of these sources contribute to atmospheric, aquatic, and terrestrial sinks, or reservoirs. It is the environmental mixing together of these sulphur compounds, aerosols, catalytic agents, and a solar energy input which induces the possible reactions listed in Table 1. Although there is little information available to date, some research is now being undertaken concerning the synergistic reactions involving harmless concentrations of $SO_2$ mixing with similar concentrations of ozone or oxides of nitrogen.

Sulphur dioxide can be reduced to $H_2S$ by bacterial processes, or it may be further oxidized to $SO_3^=$. This oxidation is dependent on ultraviolet radiation and the availability of other reactants and catalysts. The single most important factor affecting the oxidation of $SO_2$ is the amount of moisture present in the form of water droplets (Rx 10) as mist or fog. Excited $SO_2$, formed by the absorption of near-ultraviolet radiation, will react with $O_2$ forming $SO_3^=$ (Rx 12). Sulfurous acid ($H_2SO_3$) is formed when $SO_2$ is dissolved in fog or cloud droplets. This acid rapidily reacts with the dissolved oxygen to form $H_2SO_4$ (Rx 10, 19). Sulphuric acid may also be formed when $SO_2$ is oxidized to $SO_3^=$, which immediately reacts with available water vapor (Rx 14). This acid may be

neutralized by reacting with atmospheric ammonia, forming ammonium sulphate or bisulphate, or reacting with sodium chloride producing hydrogen chloride gas and sodium sulphate.

One aspect of this which is now being extensively studied is the reoccuring "acid rain". This is a term describing the increased acidity of rain and snow recorded over the past 20 years. Its environmental impact has been evidenced as vegetation damage, numerous fish kills in streams and lakes, and suggested human health adversity to acid aerosols. Evidences of "fossil precipitation" in glacial ice have recorded a pH of slightly more than 5.0. Gaseous carbon dioxide dissolved in water produces a slightly acidic solution, pH 5.6. Local studies throughout the United States and Scandinavia (Brosset, 1973; Likens, 1976) have recorded areas with precipitation pH values from 4-7 annually, 2-3 for individual storms. The highest acidity is in the highly industrialized and populated Northeastern U.S. The lowering of the pH has been attributed to the presence of strong acids, such as sulfuric, nitric, and chydrochloric (Likens, 1976).

Freshwater lakes and streams appear to be more susceptible to a lowering of pH due to sulphate and $H_2SO_4$ deposition than marine bodies of water. Seawater, with its higher salt content, and higher concentration of metals and their oxides, tend to neutralize the added acids, rapidly oxidizing the absorbed $SO_2$.

Not only are aquatic habitats, but vegetation is also, affected by the acidic precipitation. From reports to the International Conference on the Effects of Acid Precipitation held in Telemark, Normay, 1976, the following effects were noted: (1) an increased leaching of inorganic nutrients and organic substances from foliage and the soil, (2) the

acceleration of cuticular erosion of leaves, causing leaf damage when the pH was less than 3.5, (3) alteration of the plant response to pathogens, symbionts, and saprophytes, (4) altered germination of conifer seeds and seedling development.

One of the most prominent biogeochemical atmospheric reactions due, in part, to sulphur compounds is the condition known as smog. "Smog" is a contraction of the words smoke and fog, and is any air pollution event which is accompanied by a decrease in visibility. There are two types of smog, differing in their causal agents, effects on life, and chemical reactions. The London or "classical" smog is due to the accumulation of pollutants from industrial and residential combustion of fuel. Its principal pollutants are sulphur oxides and particulate matter. It generally occurs in cold, damp weather, usually getting worse at night and can build up and last for days. London smog acts as a reducing agent on material it contacts, and causes severe throat and lung irritation in humans.

Los Angeles smog is also called "photochemical smog", as it is formed through a complex series of chemical reactions involving sunlight. Its pollutant components are oxides of nitrogen, carbon monoxide (CO), ozone ($O_3$), and hydrocarbon compounds derived mainly from motor vehicle combustion engines. Normally emitted oxides of nitrogen engage in a cyclic pattern. Most $NO_x$ emitted is NO, with a small portion of $NO_2$. This $NO_2$ is sensitive to absorption of ultraviolet light, and the following reaction is the photolytic cycle of nitrogen:

$$NO_2 + \text{UV light} \longrightarrow NO + O$$

$$O + O_2 \longrightarrow O_3$$

$$O_3 + NO \longrightarrow NO_2 + O_2$$

When hydrocarbons are present, they are competitive with the $O_2$ and NO for the available oxygen (O). The result is that all of the NO is converted to $NO_2$, there is an increase in $O_3$ concentration, and the formation of hydrocarbon oxidation products such as aldehydes, ketones, and peroxyacyl nitrates. Unknown at present is the exact involvement of $SO_2$, although it is known that $SO_2$ (gas) is converted to $SO_3^=$ which then forms $H_2SO_3$ (liquid). The overall effect is that the products act as oxidizing agents on material, cause eye irritation and annoy human senses, damage property and alter the ecology of the environment.

## Effects of $SO_2$ on Vascular Plants

The toxicity of $SO_2$ was first noted by German scientists in the mid-1890's. Since then, investigations have generally focused upon its effects on vascular plants. There are numerous documentations that these macroscopic effects can be classified either as acute or chronic, according to the degree of injury. Acute injury refers to such symptoms as a loss of chlorophyll, a breakdown of cells, and the appearance of necrotic tissue, all of which result from relatively short term exposures (hours or days) to high levels of $SO_2$ pollution. Chronic injury manifests itself in the development of chlorotic tissue, and decreased rates of metabolic activities, such as photosynthesis and growth, due to the relatively lower levels of pollution over an extended period of time. There is also a "long term" effect, which manifests itself over several decades, at very low or sublethal levels of the pollutant. Robinson (1970) has suggested that the acute and chronic degrees of injury are due to the direct action of the gas (or gases) or particulates on the organism, whereas the long term effects may be caused indirectly by the products of the gas or gases.

In 1923, J. Stoklasa proposed the "invisible injury theory", based on toxic gas concentrations less than those causing visible symptoms. These pollutant levels resulted in a decrease in photosynthesis, growth and yield, early senescence, accumulation of sulphates, and an increase in susceptability to disease and insects.

Whether or not the degree of injury is readily apparent, the extent of damage and the degree of incidence depends on factors other than the concentration of the pollutant. Relative humidity, soil moisture, temperature, light intensity, age and tissue exposure of the plant, and the soil characteristics are some of the other contributing factors. Thus most vascular plants are most susceptible to $SO_2$ damage during the late spring and early summer, daily between 10 A.M. and 2 P.M. Middle-aged leaves are more apt to be affected than young or old leaves, probably related to the chemical changes associated with leaf maturation.

Acute $SO_2$ injury in broad-leafed vascular plants manifests itself as bifacial lesions, either marginal or interveinal, forming localized areas of necrotic tissue. Initially the area appears water-soaked, flaccid, later becoming ivory or red-brown in color if anthocyanins are present. Membrane degradation allows diffusion of the chlorophyll from the chloroplasts, and a bleaching process destroys the green pigmentation. In conifers, acute injury appears as apical, medial or basal bands of orange-red tissue on the needles of the current year, along with a shrinkage of tissue. These needles will abscise in 1-2 years, rather than the normal 3-5 years.

Chronic injuries in broad-leafed plants exhibit chlorotic or yellowing tissue beginning on the lower surfaces. In conifers, the older needles will exhibit a yellow-green color, later turning a reddish-brown at the tip which then continues towards the base of the leaf. In all vascular plants, chronic injury may be temporary or permanent, depending on the dosage and exposure time to the pollutant.

At the cellular level, anatomical studies have shown that palisade tissue cells shrink and collapse, reducing the leaf thickness prior to

any external symptoms. The first cells to be damaged are in the spongy mesophyll, closest to the lower epidermis. In the palisade and spongy parenchyma layers, the chloroplasts disintegrate, dispersing the chlorophyll into the cytoplasm. The protoplasts become spongy, plasmolysis occurs, and the cell walls become distorted but retain their plasmodesmata. Vascular tissues are the most tolerant of any leaf tissue.

Sulphur dioxide has the unique ability to act as a reducing and an oxidizing agent, depending on the pH of the medium in which it exists. (Table 1, Rx 11). The biochemical effects are poorly understood, and little detailed knowledge is available concerning the phytotoxicity and metabolic effects of $SO_2$.

At the receptor level (Fig. 2), most $SO_2$ enters the leaf via the stomates. Although there is no direct correlation between $SO_2$ injury and the degree of stomatal opening, nor between susceptability related to the number of stomates, any environmental factor affecting stomatal action will also affect $SO_2$ intercellular exposure.

In 1903, Haselhoff and Lindau suggested that the gases were mixed with plant aldehydes and sugars, whose products released $H_2SO_3$ and $H_2SO_4$, and that the degree of injury was modified by the carbohydrates present. Linzon (1969) stated that chronic injury is due to an accumulation of sulphate above the biochemical threshold of tolerance of the cell. The highly toxic sulphite formed through absorption of the gas is oxidized to a less toxic sulphate at about the same rate as the gas is absorbed.

Several studies (Dorries, 1932, Rao and LeBlanc, 1965; Coker, 1967; Nash, 1973) have advanced the concept that the acidity resulting from the $SO_2$ absorption decomposes the chlorophyll molecule, creating pheophytin and free Mg ions. When treated with a weak acid, the chlorophyll

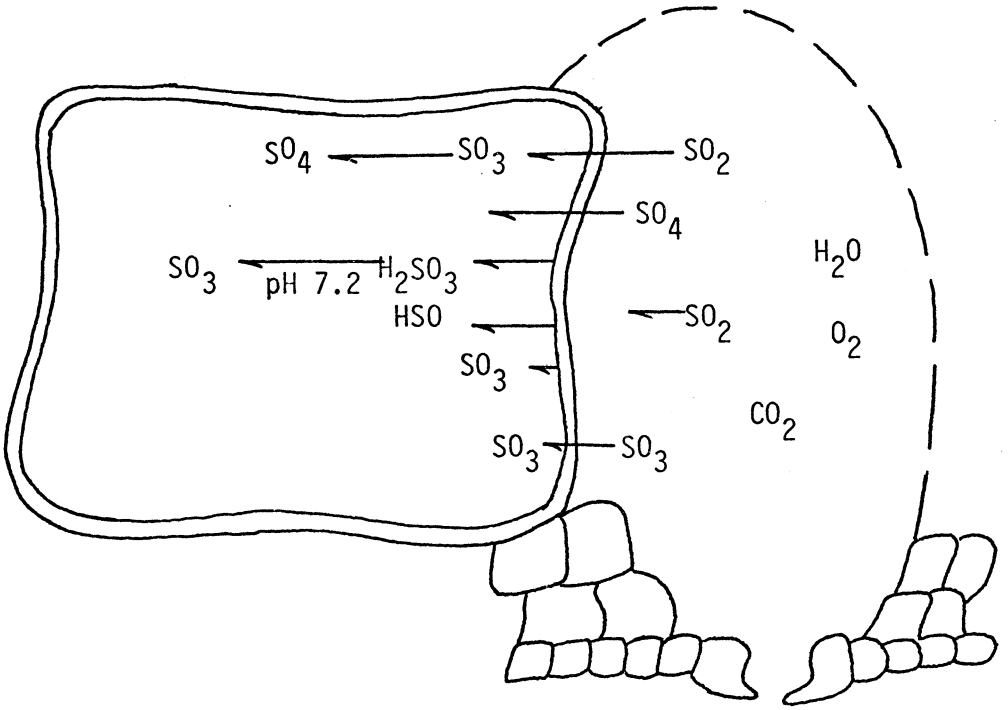



Fig. 2. Intercellular reactions involving sulphur compounds.

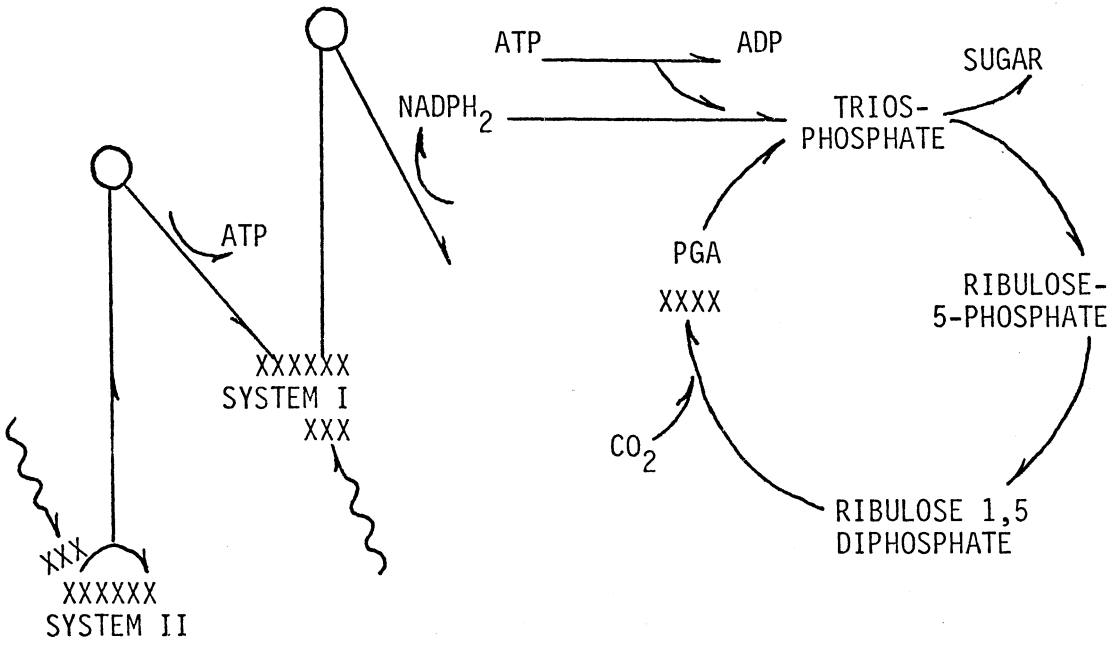



Fig. 3. Possible points of $SO_2$ interference in photosynthesis and carbohydrate synthesis.

molecule (Fig. 4) will exchange its Mg for two H, producing pheophytin. Arndt (1971) found that the destruction of chlorophyll was not $SO_2$ specific, but could also be caused by HF or HCl. He did find that the beta-carotene pigment concentration responded directly to the various levels of pollutants. Malhotra and Docking (1975) showed that at concentrations of 10-100 ppm, aqueous $SO_2$ had no effect on the concentration of chlorophyll a or b, or pheophytin. At higher concentrations (250-500 ppm) $pheophytin_a$ increased its concentration, but not $pheophytin_b$, suggesting a conversion of $chlorophyll_a$ only.

At the cellular level (Fig. 2), $SO_2$ may be transported through the membrane, and it may also react with the moisture on the membrane, forming $H_2SO_3$, $HSO_3^-$, or $SO_4^=$. The uptake of $H_2SO_3$, $HSO_3^-$, and/or $SO_2$ is more rapid than the more highly charged $SO_3$ and, following their dissociation in the cell, results in an acidification of the cell and an accumulation of $HSO_3^-$ and $SO_3^=$ ions (Sundstrom and Hallgren, 1973). The normal 7.2 pH of the plant cell is not immediately affected by the $SO_2$ due to a natural buffering capability. In the late stages of acute absorption, when the change in pH is greater than the buffering capacity, there is a coagulation of the cell plasma protein. Sulphur dioxide has been shown (Wellburn et al, 1972) to interfere with the permeability and structure of cell membranes, and with their enzymatic activity, thus affecting many cellular biological processes, as well as with the photosynthetic pigments.

Cell membranes function as regulatory mechanisms and as sites for many biochemical reactions. Their selective permeability is due to their composition, structural orientation and chemical properties. The $SO_2$ interference noted by several studies (Thomas, Hendricks and Hill, 1950; Wellburn et al, 1972) affects numerous biochemical processes.

Hyatsu and Miller (1972) showed that sulphite can react with cellular free radicals, which might lead to the splitting of phosphodiester linkages in DNA molecules. Again in 1972 (Shapiro and Braveman) demonstrated that one type of $SO_2$ toxicity might arise from the inactivation of DNA or mRNA. Another concept is that disulphide bonds found in proteins can be cleaved by bisulphites (Bailey and Cole, 1959; Cecil and Wake, 1962), resulting in an enzyme deactivation due to an altered tertiary structure. The studies of Cecil and Wake (1962) reported that $SO_2$ inactivated many enzyme systems by splitting their disulphide linkages, while activating some hydrolytic enzyme systems, possibly through confirmational changes.

Malhotra and Hocking (1975) concluded that at low concentrations, under prolonged exposure, the pollutant stimulated some enzyme systems, i.e., chlorophyllase (Fig. 2). At higher concentrations, there was a total senescence by inhibition of chlorophyllase activity, and a changing of chlorophyll to pheophytin. They also noted a loss of photosynthetic activity through the competition between $SO_2$ and $CO_2$ for the active site on ribulose-1-5-diphosphate (RuDP) carboxylase. This agrees with Ziegler (1972) who detected competition between $SO_3^=$ and $CO_2$, the natural substrate for RuDP carboxylase. At low concentrations of $SO_3^=$, there was competitive inhibition of the enzyme with respect to RuDP and Mg ions. It has been shown that at intermediate levels of $SO_2$, there is a decreased rate of photosynthesis prior to any visible damage (Showman, 1972; Turk and Wirth, 1975).

In 1929, Noack believed that $SO_2$ injury to vegetation was characterized by the inactivation of the iron in the chloroplasts, interfering with its catalytic properties in assimilation. Secondary photochemical

oxidative processes then caused bleaching and subsequent death of the cells. Only recently, when the ultrastructure of the chloroplasts could be studied, has there been any information on the effects of $SO_2$ at a sub-cellular level. Wellburn et al (1972) reported a reversible swelling of the thylakoid within the chloroplasts when broad bean plants were fumigated with $SO_2$ (0.25 ppm, 2 hours). Thylakoids contain some of the dark reaction enzymes in their lumen, and have photophosphorylation particles on their interconnections between membranes (Howell and Moudriankis, 1967) and any disruption to these structures will affect $CO_2$ assimilation.

Compounds of $SO_2$ then generally interact with plant metabolic activities (Fig. 2) by: (1) changing and degrading pigments, thereby slowing the production of ATP, $NADPH_2$, and the fixation of $CO_2$, (2) the disorientation of chloroplast membrane and (3) inhibition of enzymes.

## Effects of $SO_2$ on Nonvascular Plants

Of the nonvascular plants, lichens have been used most extensively as pollution indicators, primarily because of their ability to accumulate foreign substances even in dilute concentrations. Many references to lichen vegetation studies in polluted areas are listed in Air Pollution and Lichens (Ferry et al, 1973). Sulphur dioxide pollution studies have focused on epiphytic cryptogams (LeBlanc, 1967; Johnson and Sochting, 1976) or bryophytes (Coker, 1968; Gilbert, 1968; Taoda, 1973) or both lichens and bryophytes (Syke, 1968, LeBlanc and Rao, 1973; Turk and Wirth, 1975).

The disappearance of mosses from cities was first recorded by Nylander in 1866 for the Jardins du Luxembourg in Paris. In 1892, Arnold noted the same occurrence in the city of Munich. Since then, the reduction in epiphytes and bryophytes has been recorded in New York (Brodo, 1961), Stockholm (Skye, 1968), Montreal (Desloover and LeBlanc, 1970), Montana (Sheridan et al, 1976) and Newcastle, England (Gilbert, 1968). Lately, vegetational studies have been concerned with a particular point source of pollution (LeBlanc and Rao, 1966; Rao and LeBlanc, 1967; Newberry, 1974; Sheridan et al, 1976). Additional studies are listed in Table 3.

All of these studies, whether urban or point source, establish zones which have a pollutant level influencing the vegetation. The zone least influenced by the pollutant is characterized by normal growth forms, morphology, and ecological succession and the largest species diversity, population, distribution and biomass. As the central zone nearest the

Table 3. Literature Available Concerning Air Pollution Study Areas

| Area | Date | Literature |
|---|---|---|
| Merseyside, Eng. | 1976 | Bevan, R.J. Ph.D. thesis, Univ. Liverpool |
| Paris, Fr. | 1977 | Dervelle, S. Revue bryol. lichen 43:137-158 |
| Mecklenburg, Ger. | 1976 | Doll, R., Z ges. Hygiene Grenzg. 11:840-43 |
| Alberta, Can. | 1976 | Douglas, G.W. & A.C. Skorepa, Environ. Research Monogr. 1976-2 |
| Poland | 1977 | Grodzinska, Vegetation Science and Environmental Protection. |
| Kokkola, Finld. | 1977 | Laaksovirta, K. and H. Olkkonen, Annls. bot. fenn. 14:112-130 |
| Edmonton, Can. | 1973 | Lee, T., and D.H. Vitt, Proc. of Workshop on Sulphur Gas Research in Alberta |
| Scotland | 1975 | O'Hare, G.P., J. Biogeography 1:135-146 |
| Christchurch, N. Zea. | 1970 | Daly, G.T., Proc. N.Z. ecol. Soc. 17:70-79 |
| Sudbury, Can. | 1973 | LeBlanc & Rao, Ecology 54:612-617 |
| Idaho & Wash. | 1974 | Hoffman, G.R. Environ. Pollut. 7:283-301 |
| Wisconsin | 1974 | Newberry, G., Bryologist 77:561-576 |
| Ohio | 1975 | Showman, R.E. Bryologist 78:1-6 |
| Nashville, Tenn. | 1972 | Mathis & Tomlinson, J. Tennessee Acad. Sci. 47:67-73 |
| Quebec, Can. | 1974 | LeBlanc, Robitaille & Rao, J. Hattori bot. lab. 38:405-433 |
| Winnipeg, Can. | 1974 | Stringer, P.W. & M.H.L. Stringer, Bryologist 77:405-426 |

pollutant source is approached, the studies noted above document a decrease in species density, diversity and number, as well as displaying an altered morphology, physiology, reproductive capability, and reduced biomass. Sernander (1926) referred to this central zone as an epiphytic desert, characterized by either an absence of species or an environmentally induced modification of those few species present, i.e., small, compact colonies with low cover, which are often sterile.

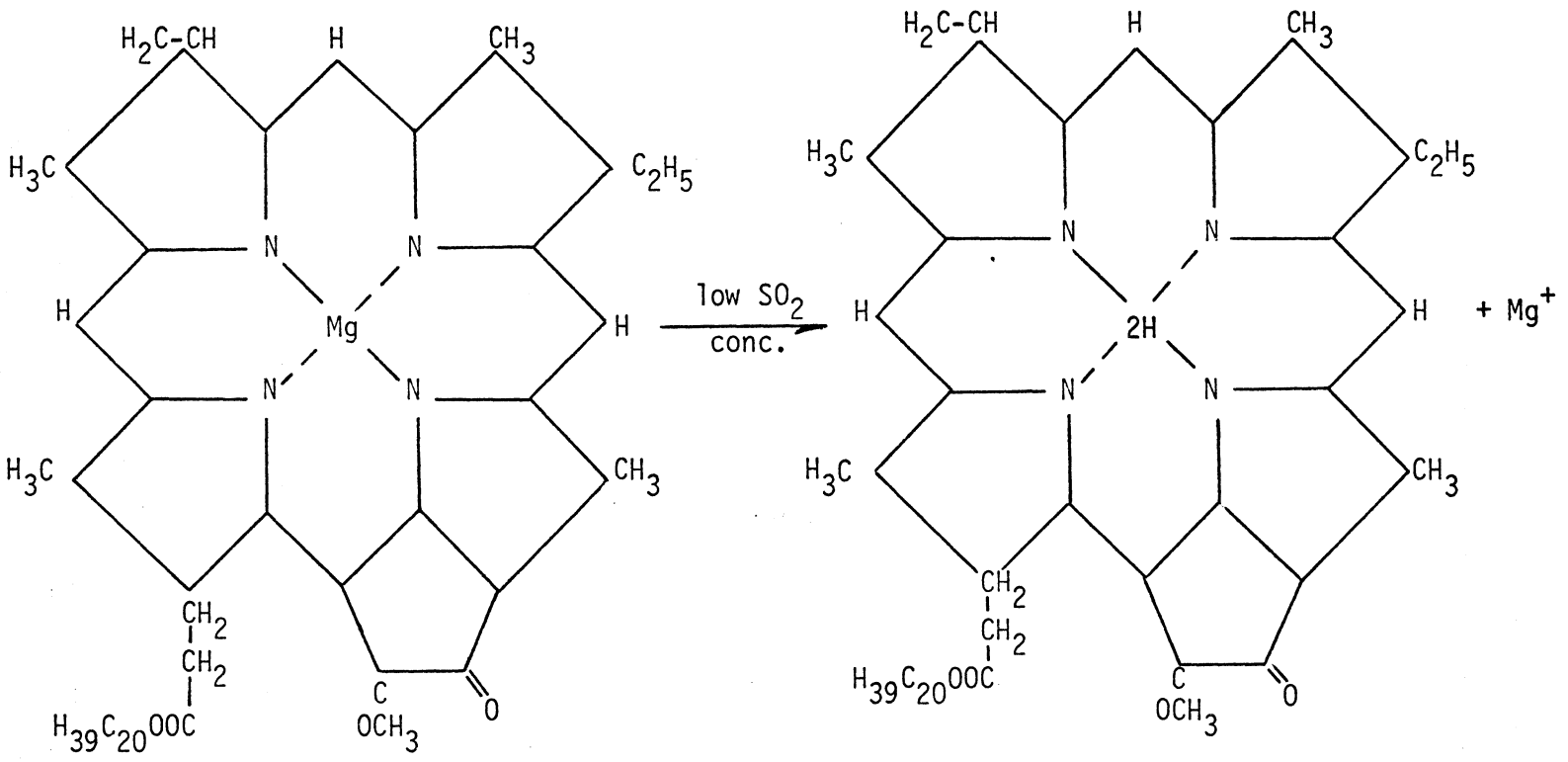
The cause of these epiphytic deserts remains to be resolved. It may be due to the reduced light intensity in urban areas or to the mechanical action of man. Other possibilities are altered temperature and humidity, since microclimates in urban areas tend to have higher temperatures and reduced relative humidity in comparison to rural microclimates (Gilbert, 1965). Likewise, drought (the lack of dew or wet fog) and air pollution, both present in cities, may be important factors, as reported by Barkman (1968). It may be that the absence or decline of plant species is due to pollutant influences upon the reproductive phases of the cryptogams, for Gilbert (1968) and Nash (1974) reported a greater pollutant sensitivity in protonema than in mature gametophytes. While Gilbert attributed the epiphytic deserts to the tendency of species sterility (no spore production) as the central zone is approached, ultimately resulting in sterile populations, Nash III believes the deserts are due to a block in the moss reproduction, namely the protonemal stage. The actual cause may be a combination of factors stemming from, and influenced by, the environment.

According to Barkman (1973) and Sundstrom and Hallgren (1973), bryophytes have a higher $SO_2$ sensitivity than vascular plants because (1) they lack an impermeable cuticle and regulatory stomata, (2) they

absorb rain water directly over their entire surface, whereas vascular plants obtain most of their water indirectly after it is filtered through the soil, and (3) bryophytes are most active in the seasons of higher humidity, spring, fall and winter. Winter is the season with the highest pollution levels of the year, due to a lower formation of clouds, frequent temperature inversions and fogs, and extensive residence heating.

Lichens and bryophytes are similar in their responses to $SO_2$ pollution, exhibited during fumigation and thalli transplant studies. Rao and LeBlanc (1966) found that Xanthoria algal cells, after 5 ppm $SO_2$ exposure for 24 hours, exhibited bleached chlorophyll, brown spots on the chloroplasts, a permanent plasmolysis of the cells, and an abnormal $Mg^{+2}$ ion and pheophytin content. They attributed the bleached chlorophyll to the presence of $H_2SO_3$, a strong reducing and bleaching agent. They believed plasmolysis was due to a difference in the cellular osmotic pressure created by the presence of $SO_3^-$ and $SO_4^=$. The $Mg^{+2}$ ions were the products of $chlorophyll_a$ degradation into $pheophytin_a$ under acidic conditions. Similar studies (LeBlac, 1967; Skye, 1968; Coker, 1968; Nash, 1973; LeBlanc and Rao, 1973) have shown the same morphological and physiological responses in mosses and in other lichens, with the algal component being more sensitive than the fungal component. Skye (1968) described not only discoloration in the exposed lichens, but also changes in their morphology.

Showman (1972) reported a decrease in net photosynthesis and in dark reactions with no chlorophyll damage at 6 ppm when he fumigated Cladonia symbionts. This supported Hill's conclusions (1971) that the chlorophyll degradation is a secondary effect of $SO_2$, and has little to do concerning plant (Usnea, Parmelia) disappearance in polluted areas.




Chlorophyll a

Pheophytin a

Fig. 4. Degradation of chlorophyll$_a$ to pheophytin$_a$.

Showman postulated that there is some mechanism other than chlorophyll degradation responsible for these decreases. Turk and Wirth (1975) found a decrease in photosynthesis in mosses after exposure to 15 mg $SO_2/m^3$ air, suggesting that mosses are more sensitive to $SO_2$ than lichens. In a field study, (Sheridan et al, 1976) it was found that the lichen photosynthetic rate increased after 13-19 days exposure to $SO_2$ concentrations of 0.75 ppm. It was postulated that the low $SO_2$ concentration stimulated $O_2$ production, either by the sulphide and bisulphite ions acting as electron acceptors, or by the $SO_2$ uncoupling the electron transport system from photophosphorylation (Fig. 3).

Nash (1973) found a reduction in the carbon content of moist lichens with an increase in $SO_2$ concentration. However, this reaction is not $SO_2$ specific, as $NO_2$ injury has also been noted by a decreased carbon concentration (Nash, 1973).

Cryptogam sensitivity to $SO_2$ varies among different genera and species, and with various environmental factors. The dispersion of the pollutant in the ecosystem, as well as its biogeochemical reactions regulates the chemical state and exposure to the vegetation. Gilbert (1968) noted several controlling influences of the habitat, i.e., shelter, substrate pH, buffering capacity, and nutrient flushing. He proposed that a high pH reduces the toxic effect of the pollutant directly by ionizing the sulphurous acid, or indirectly as an exclusion agent of the habitat. Lichens growing on basic substrates tolerate higher levels of pollution than those growing on acid substrata (Hill, 1971). Studies by Syratt (1963), LeBlanc (1968), and Coker (1968) indicate cryptogam $SO_2$ sensitivity to be increased under humid conditions, or if the thalli are saturated prior to fumigations (Nash, 1973). Syratt and Wanstall (1963)

found that the breakdown of chlorophyll$_a$ due to the low concentration of $SO_2$ appears to depend on the $SO_2$ concentration and the humidity: the higher the humidity, the greater the degradation of chlorophyll$_a$. They found it difficult to obtain 100% humidity in laboratory fumigations because of the increased chamber temperature caused by the high light intensities. The gases readily dissolved in the resulting condensate, altering the experimental controls.

Rao and LeBlanc (1973) established a 0.05 ppm $SO_2$ threshold concentration for lichen and moss transplants. They found chlorophyll to be $SO_2$ sensitive to concentrations in excess of 0.154 ppm, with long term levels of less than 0.002 ppm causing no injury, 0.006-0.03 ppm causing chronic injury, and greater than 0.03 ppm causing acute injury. Taoda (1973) made allowance for a time factor, with injury sustained the most at 0.8 ppm for 10-40 hours, or at 0.4 ppm for 20-80 hours. He recorded chronic injury (poor growth) at 0.2 ppm for greater than 100 hours exposure. Nash (1973) established a short term fumigation susceptability threshold for lichens at about 0.5 ppm. Nash and Nash (1974) noted that mature gametophytes were $SO_2$ resistant at concentrations of 2-4 ppm.

The purpose of this investigation is to submit selected hepatics to $SO_2$ fumigation, noting morphological alterations. It is anticipated that the liverworts, having a more humid microclimate than most mosses and lichens, would be as susceptible, if not more so, to $SO_2$ and would respond in the same manner as the previously studied cryptogams. Accurate determination of injury thresholds cannot be established by the equipment available. However the general responses to the fumigation should indicate the degree of injury.

## Materials and Methods

The following hepatics were selected as test organisms because of their frequency of occurrence, morphology, and habitat.

1. Scapania nemorosa (L.) Dum. A complicate-bilobed leafy liverwort is found in compact olive-green cushions to loose tufts on moist shaded banks or humus-covered rocks; preferring an acid substrate. The leaves are irregularly ciliate-dentate; the ventral lobe 2-2.5 times larger than the dorsal lobe, and obovate-obtuse. The dorsal lobe is reniform to ovate with a short point or rounded apex. Both lobes bear cilia with a length of 2-3 cells. The leaf cells are oval-quadrate, thick-walled with a rough cuticle.

2. Blasia pusilla L. A tholloid liverwort which has been considered to be a transition between thalloid and foliose hepatics because it is several times dichotomously branched. It is broadly ligulate, with a lobed margin, prostrate, and forms green to dark or bluish-green rosettes or tangled mats. It has small oblong, ovate or heteromorphic dentate scales in irregular rows on either side of the wide costa. Toothed underleaves are present. Subspherical organs called "leaf auricles" are located near the base of the lobe, which are soon occupied by the blue-green alga Nostoc. Its habitat is moist banks and wet clayey or gravelly soil. The leaf cells are 30-60 u in diameter, rhombic to hexagonal, with oblong marginal cells 20-30 u wide.

3. Jamesoniella autmnalis (D.C.) Steph. A leafy liverwort forming dark green or yellowish-green prostrate mats, which later becomes

a characteristic reddish-brown color. The plants (1-4 cm long) grow flat along the substrate, or in tangled mats; stem tips are often ascending. The entire leaves are oblong-oval on the lower parts of the stem, rotund-oval above, and obliquely inserted the entire length of the stem. Individual leaves are convex, the margins entire or frequently retuse, with a smooth cuticle. The leaf cells are rounded, thin-walled with small but distinct trigones. Oil bodies are present within the cells, usually 7-20 per cell. Underleaves are frequently obsolete except in the apical area, and when present are subulate. Jamesoniella is found in a wide variety of rock habitats but prefers sandstone (5.5-7.0 pH). It is often intermixed with other liverworts and mosses in shady places, or on mossy walls, humus-covered rocks, and on decaying logs (4.5-5.2 pH).

4. Lophocolea heterophylla (Schrad.) Dum. This leafv liverwort grows prostrate on decaying logs and stumps in moist woods or rarely on open soil. The stems (1-2 cm long) are variously branched, with numerous tufts or rhizoids around the small, deeply bifid underleaves. The entire plant tends to be whitish or yellowish-green, or rarely a solid green. The polymorphic leaves may be entire, bifid, or slightly notched, on the same plant.

These specimens were collected at the following sites:

a. Rocky Branch Nature Preserve (Clark Co., R12W, T12N, sec. 29)
b. Fox Ridge State Park (Coles Co., T11N, R9E, sec. 12, 13)
c. Polecat Creek (Coles Co., T12N, R9E, sec. 10)
d. Rocky Hollow (Clark Co., T9N, R12W, sec. 5)

Pure communities of the desired genera were collected and maintained up to three days in translucent plastic terraria under artificial lighting (200 foot candles, at 23°-28°C).

Prior to fumigation, specimens were divided and placed in open glass petri plates, misted with distilled water, and provided with a nutrient

solution (Voth, 1941, Table 4). A constant air supply of 1.2 liters per minute was provided to each of the Eduquip Ecological chambers (Fig. 5) by two Metaframe aquarium pumps (2.0 lpm output). Humidity was introduced into each line by bubbling the air through a flask of distilled water. Charcoal inline filters purified the air prior to gas introduction from a 1.0 liter lecture bottle of $SO_2$. The liverworts were subjected to various concentrations of $SO_2$ for 8 hours, under 180 foot candles illumination. The gas sampling using Kitagawa $SO_2$ low-range detection tubes was accomplished during the last half-hour of fumigation. After fumigation, the specimens were removed, the gas supply shut off, and the chamber was exposed to two hours of continued air supply to remove residual gas.

Chlorophyll analysis involved removing the liverworts from the substrate, washing them in distilled water, and weighing each species on a Sartorius analytical balance. Extraction of the chlorophyll involved maceration of the plant tissue, using a mortar and pestle in several ml of 80% acetone. A very small amount of $CaCO_3$ was added to prevent plant acids from destroying the chlorophyll. The grindate was filtered using Whatman filter paper in a Buchner funnel with a water aspirator. The filtrate was made up to 25.0 ml with 80% acetone and refrigerated. The chlorophyll analysis was performed using a Beckman Acta spectrophotometer, visible range. Determination of chlorophyll in solution was based on MacKinney (1941) and Arnon (1949).

Table 4. Nutrient Solution for Artificial Growth of Liverworts (Voth, 1941)

| | | | | |
|---|---|---|---|---|
| Plants require: | K | at 1.2 mM | $NO_3$ | at 3.4 mM |
| | Ca | 0.7 mM | $PO_4$ | 0.4 mM |
| | Mg | 1.4 mM | $SO_4$ | 0.8 mM |

Make up 0.5 M solutions of each

Final solution requires:

| | |
|---|---|
| $KNO_3$ ..... | 1.6 ml |
| $Ca(NO_3)$ .... | 1.4 ml |
| $Mg(NO_3)_2$ ... | 1.2 ml |
| $KH_2PO_4$ ..... | 0.8 ml |
| $MgSO_4$ ..... | 1.6 ml |

Add distilled $H_2O$ to make up 1.0 liter

Solution pH of 6.85

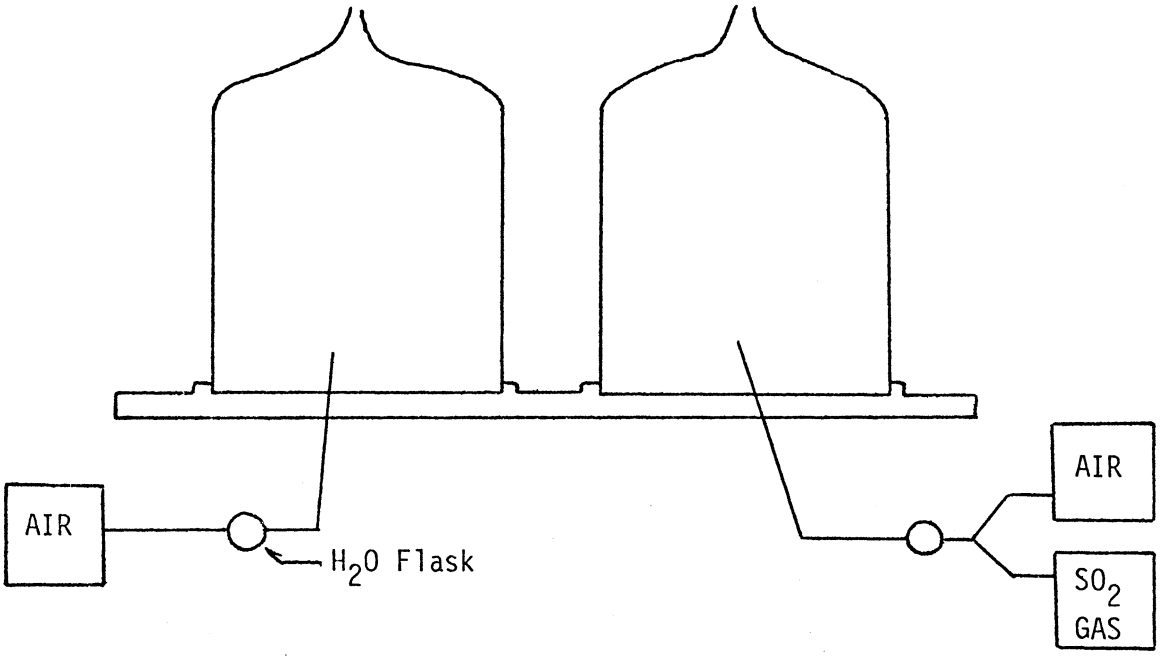



Fig. Diagram of fumigation equipment.

## Results and Discussion

The general morphological response of all fumigated organisms was an overall chlorosis of plant tissue, appearing more prominent at higher pollutant levels. This discoloration appeared to be dosage-time related; preliminary fumigations of 20-50 ppm $SO_2$ produced chlorosis in the second or third hour, with chlorosis appearing later in time as the $SO_2$ concentration was decreased. In the experimental range (less than 1.0 ppm), chlorosis became evident in the seventh or eighth hour (0.6 and 0.4 ppm) or was unapparent (0.1-0.2 ppm). Chlorotic areas were widespread in those organisms having much of the plant thallus exposed (Blasia, Lophocolea). In those genera which grew entangled or in dense mats (Scapania, Jamesoniella), chlorosis was more pronounced in the exposed plant parts, including the erect perianths. There appeared to be no plasmolysis of the cells in microscopic examination of the leaf cells. This was as expected, as plasmolysis occurs at higher concentrations (5 ppm or greater) or over a longer exposure period.

Preliminary chlorophyll analysis data was obtained by extracting chlorophyll from a 0.203-0.001 gram sample of fresh, debris-free plant tissue. This established Lophocolea as having the highest concentration of total chlorophyll and chlorophyll$_a$, Scapania having the least (see Table 5). In all genera, chlorophyll$_a$ comprised 34-37% of the total chlorophyll concentration extracted.

Absorption spectral analysis of the chlorophyll extractions (pg. show a decrease in chlorophyll content of the fumigated plants with respect

to the nonfumigated or control plants. Previous studies (Dorries, 1932; Rao and LeBlanc, 1965; Coker, 1967; Nash, 1973; Sundstrom and Hallgren, 1973) indicate that chlorophyll$_a$, when subjected to a weak acid or a reducing pollutant such as $SO_2$, is degraded to pheophytin$_a$ and free Mg ions. There was no detectable change in the pH of the nutrient solution, nor in a distilled water sample within the fumigation chamber. It must be surmised then that the degradation was due to the absorption of the $SO_2$ gas or of its acidic product.

There appears to be no direct relationship between the extent of chlorophyll loss and the amount of $SO_2$ introduced, although at very low concentrations the response of the plants appeared less affected than at higher concentrations. From Table 5, the loss of total chlorophyll is directly traceable to a similar loss of chlorophyll$_a$, indicating that the $SO_2$ or its product is specific for the yellow-green chlorophyll.

Most fumigated thalli were similar in their chlorophyll loss. However Blasia, with its associated alga, had a marked difference in loss. To determine the effect of $SO_2$ on Nostoc alone, a pure laboratory culture of this algal genus was fumigated at 0.4 ppm and extracted in 80% and 100% acetone. The 100% acetone extraction procedure was to retain the water soluble phycobilins present in Nostoc, as well as the chlorophyll$_a$.

In 80% acetone, Nostoc also experienced about a 35% reduction in total chlorophylls, with a 22% loss of chlorophyll$_a$. These calculations (Arnon, 1949; MacKinney, 1941) do not seem to hold true for extractions using 100% acetone, as they calculate a net gain during fumigation. A problem arises here which has yet to be resolved. Plant chlorophylls are experimentally extracted using 80% acetone. In contrast, algal chlorophylls are best extracted using hot or cold methanol (Strain, 1958). There is no

available correlation data or conversion mathematics to equate the two extraction procedures. To date, no chlorophyll studies have been updated involving a plant and algal association. It is apparent that Nostoc does experience a chlorophyll breakdown and discoloration of the colonies. But to relate its loss, and its total effect on the chlorophyll loss of Blasia, is difficult at this point.

All organisms tested exhibited a discoloration and substantial loss of total chlorophyll and chlorophyll$_a$ at 0.4 ppm. These hepatics are, at the least, equal in sensitivity to previously tested cryptogams. At the best, they may be more sensitive, but because of their varying substrate, habitat requirements, and restrictive occurrence, they may not prove as a readily available indicator of $SO_2$ pollution.

Table 5. Chlorophyll Computations of $SO_2$ Fumigated Liverworts

| Genus | Conc. $SO_2$ | $X_1$ | Chl.$_{T-2}$ | Chl.$_{T-3}$ | Chl.$_{a-1}$ | Chl.$_{a-2}$ |
|---|---|---|---|---|---|---|
| Lophocolea | 0.00 | C | 1.230 | | .775 | |
| Scapania | 0.00 | C | .656 | | .417 | |
| Blasia | 0.00 | C | .820 | | .512 | |
| Jamesoniella | 0.00 | C | 1.030 | | .678 | |
| Jamesoniella | 20 | C | .533 | | .343 | |
| | 20 | F | .310 | 41.8 | .199 | 42.0 |
| Blasia | 20 | C | .471 | | .321 | |
| | 20 | F | .258 | 45.2 | .200 | 37.7 |
| Scapania | .6 | C | .338 | | .216 | |
| | .6 | F | .149 | 55.9 | .097 | 55.1 |
| Blasia | .6 | C | .351 | | .067 | |
| | .6 | F | .266 | 24.2 | .050 | 25.4 |
| Jamesoniella | .6 | C | .402 | | .095 | |
| | .6 | F | .166 | 58.8 | .045 | 52.6 |
| Lophocolea | .6 | C | 1.15 | | .75 | |
| | .6 | F | .60 | 47.8 | .33 | 56.0 |
| Blasia * | .6 | C | .425 | | .273 | |
| | .6 | F | .405 | 5.1 | .255 | 6.6 |
| Nostoc * | .4 | C | .176 | | .149 | |
| | .4 | F | .347 | | .253 | |
| Nostoc | .4 | C | .128 | | .092 | |
| | .4 | F | .084 | 34.4 | .072 | 21.7 |
| Jamesoniella | .1-.2 | C | .342 | | .227 | |
| | .1-.2 | F | .253 | 26.0 | .196 | 13.7 |

1 C = control
F = fumigated

2 in mg Chl./l. sol./g.f.wt.

3 % change

* extracted using 100% acetone

Graph 1.

Absorption spectra of chlorophylls for:

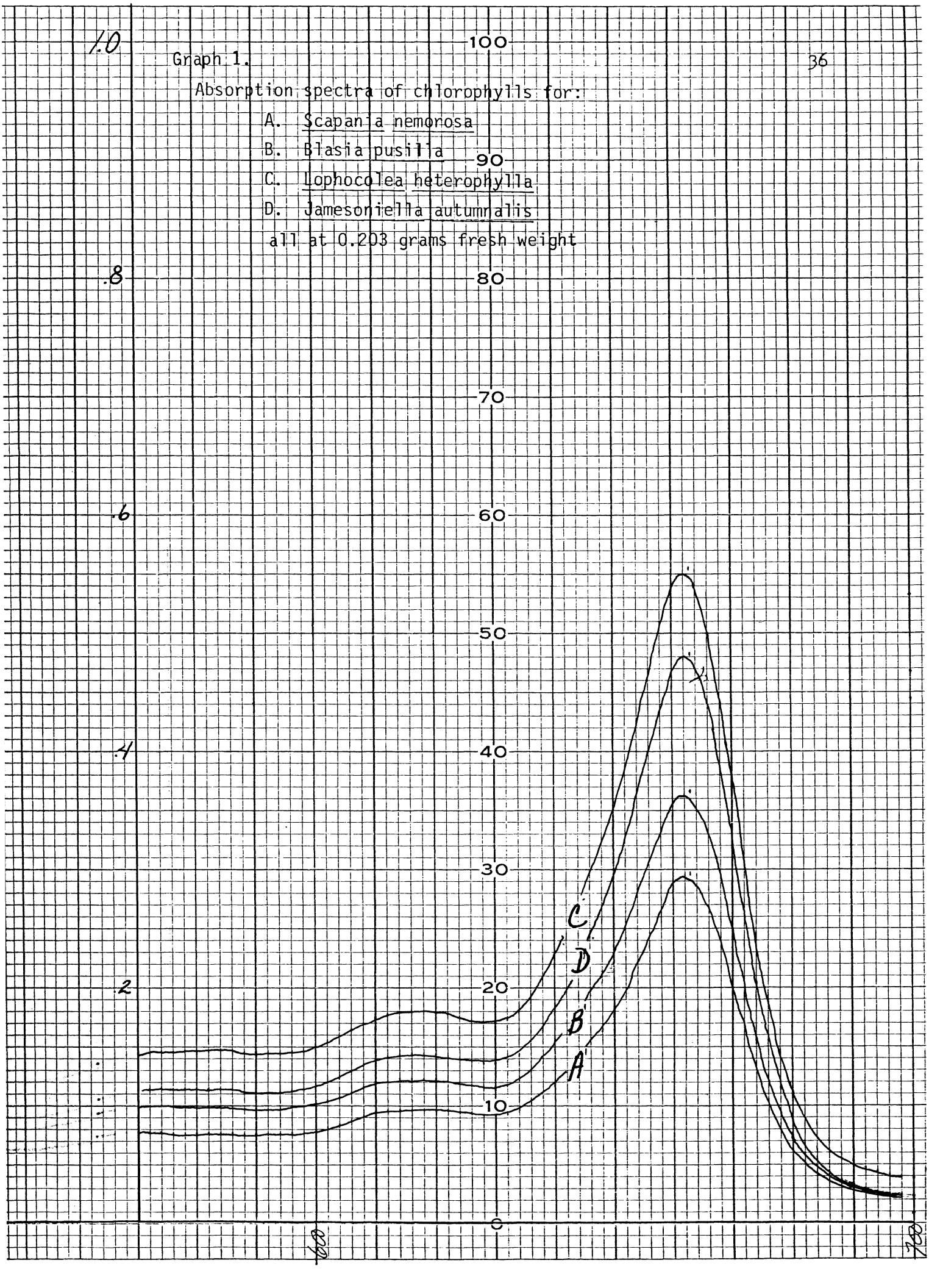
A. *Scapania nemorosa*

B. *Blasia pusilla*

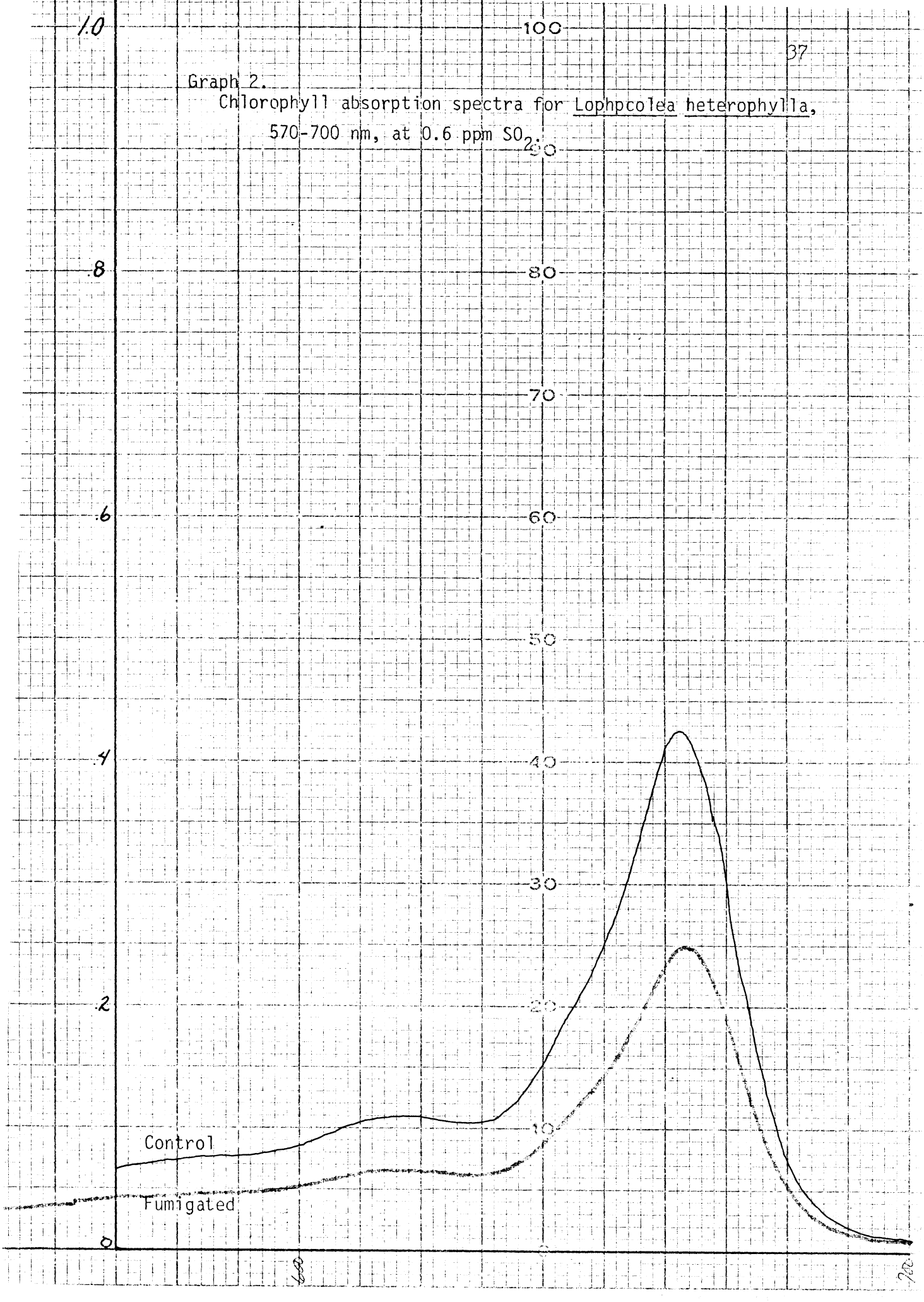
C. *Lophocolea heterophylla*

D. *Jamesoniella autumnalis*

all at 0.203 grams fresh weight

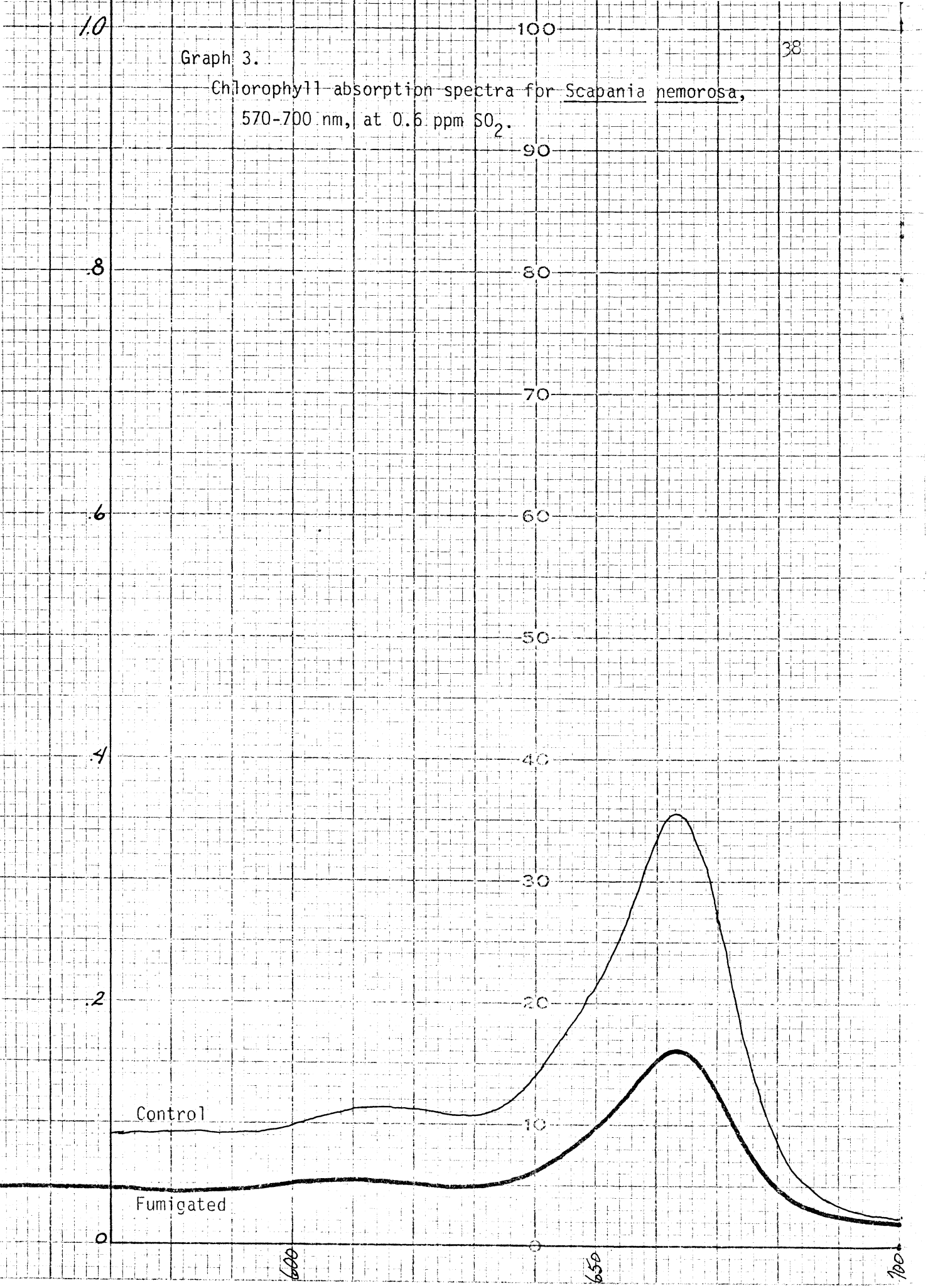


Graph 2.
Chlorophyll absorption spectra for Lophpcolea heterophylla, 570-700 nm, at 0.6 ppm $SO_2$.



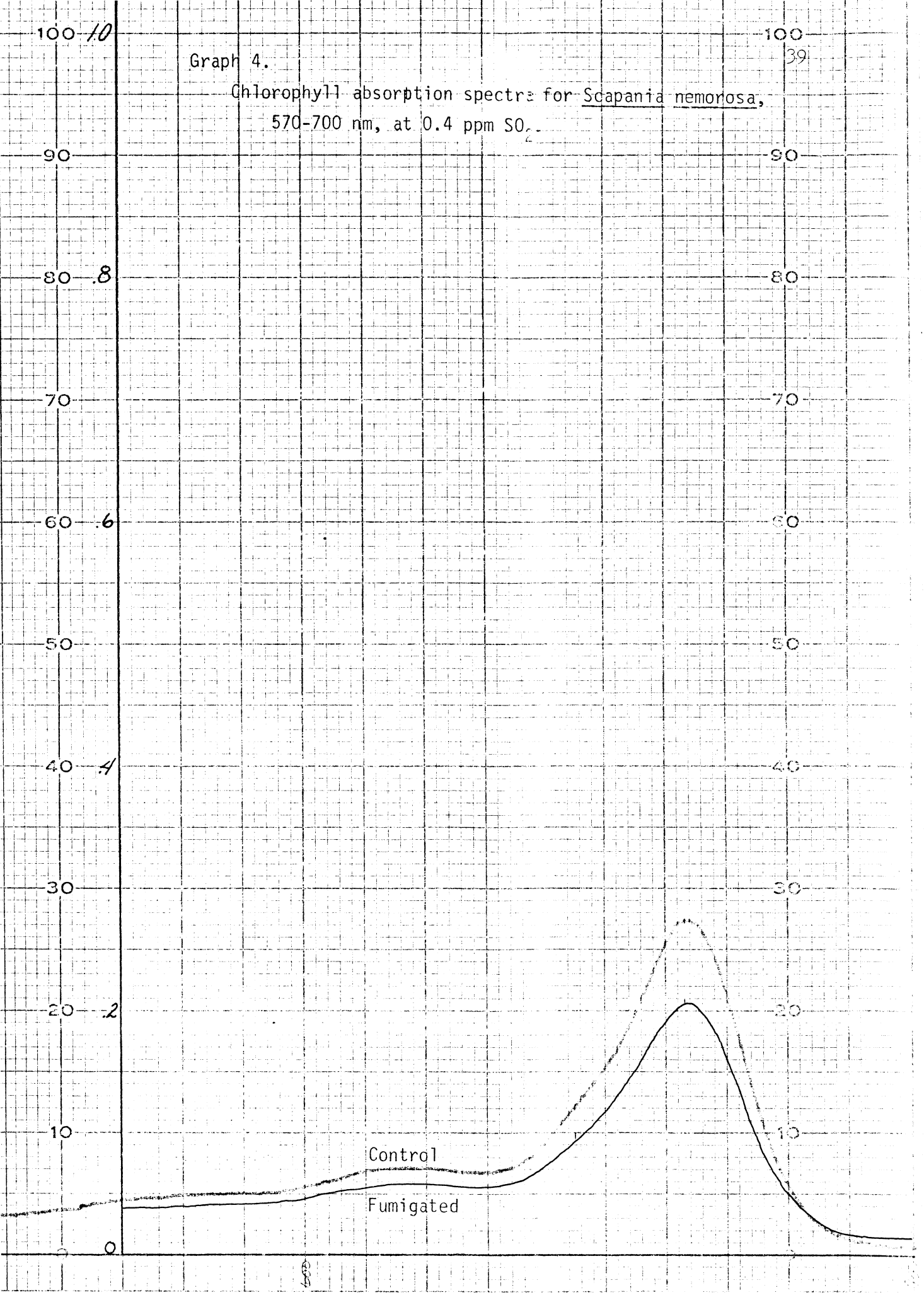
Graph 3.

Chlorophyll absorption spectra for Scapania nemorosa, 570-700 nm, at 0.6 ppm $SO_2$.



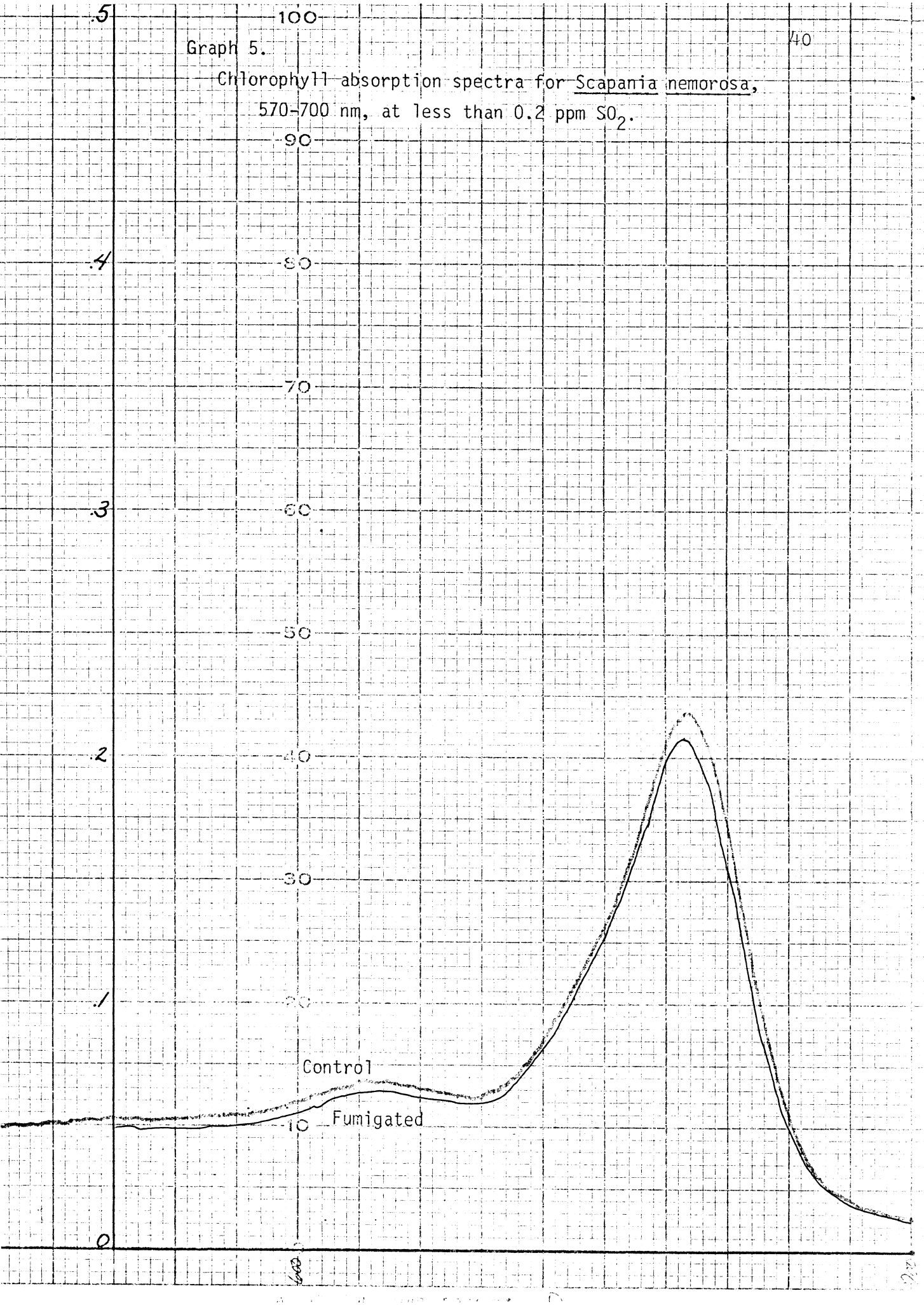
Graph 4.

Chlorophyll absorption spectra for Scapania nemorosa, 570-700 nm, at 0.4 ppm $SO_2$.



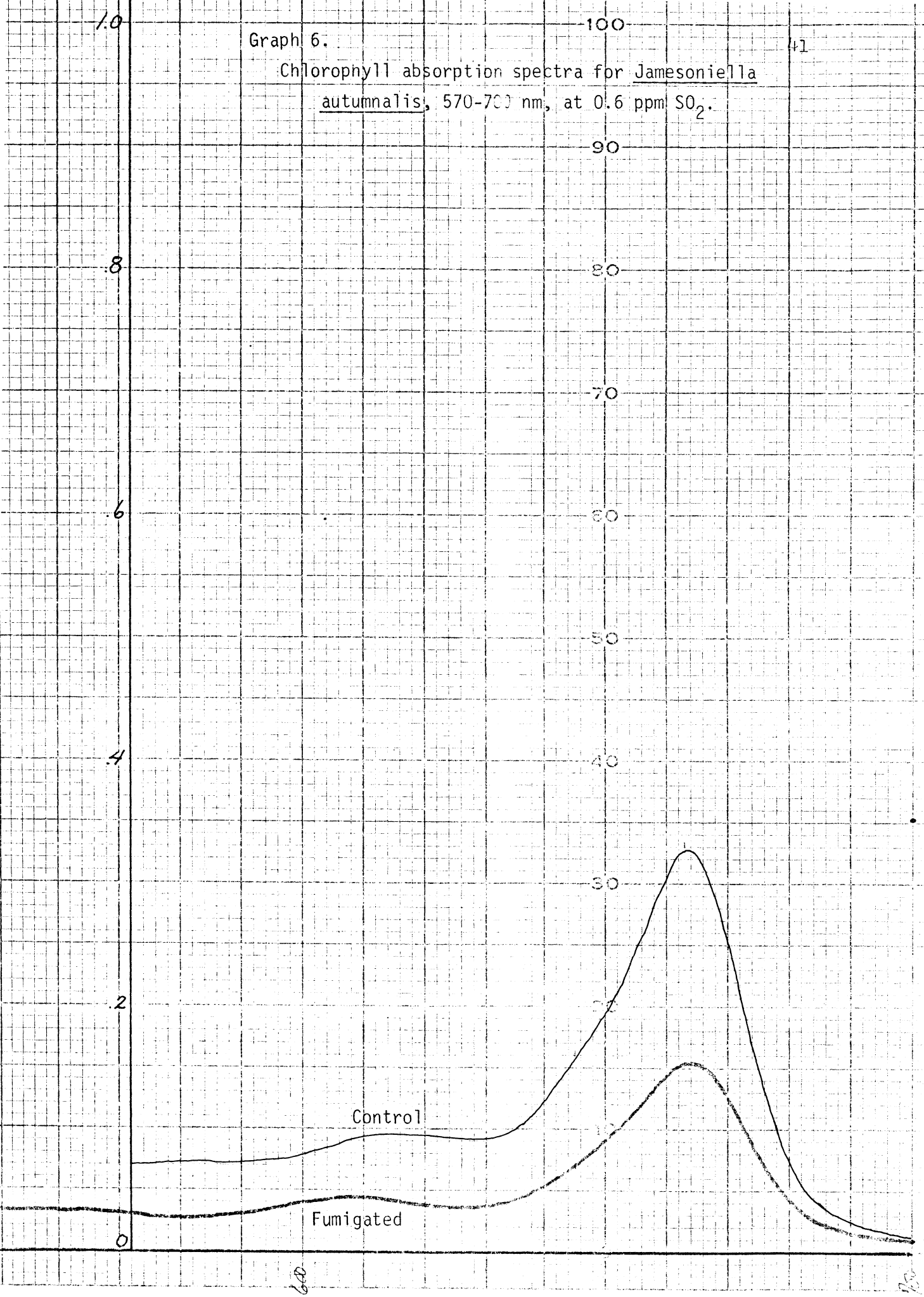
Graph 5.

Chlorophyll absorption spectra for Scapania nemorosa, 570-700 nm, at less than 0.2 ppm $SO_2$.



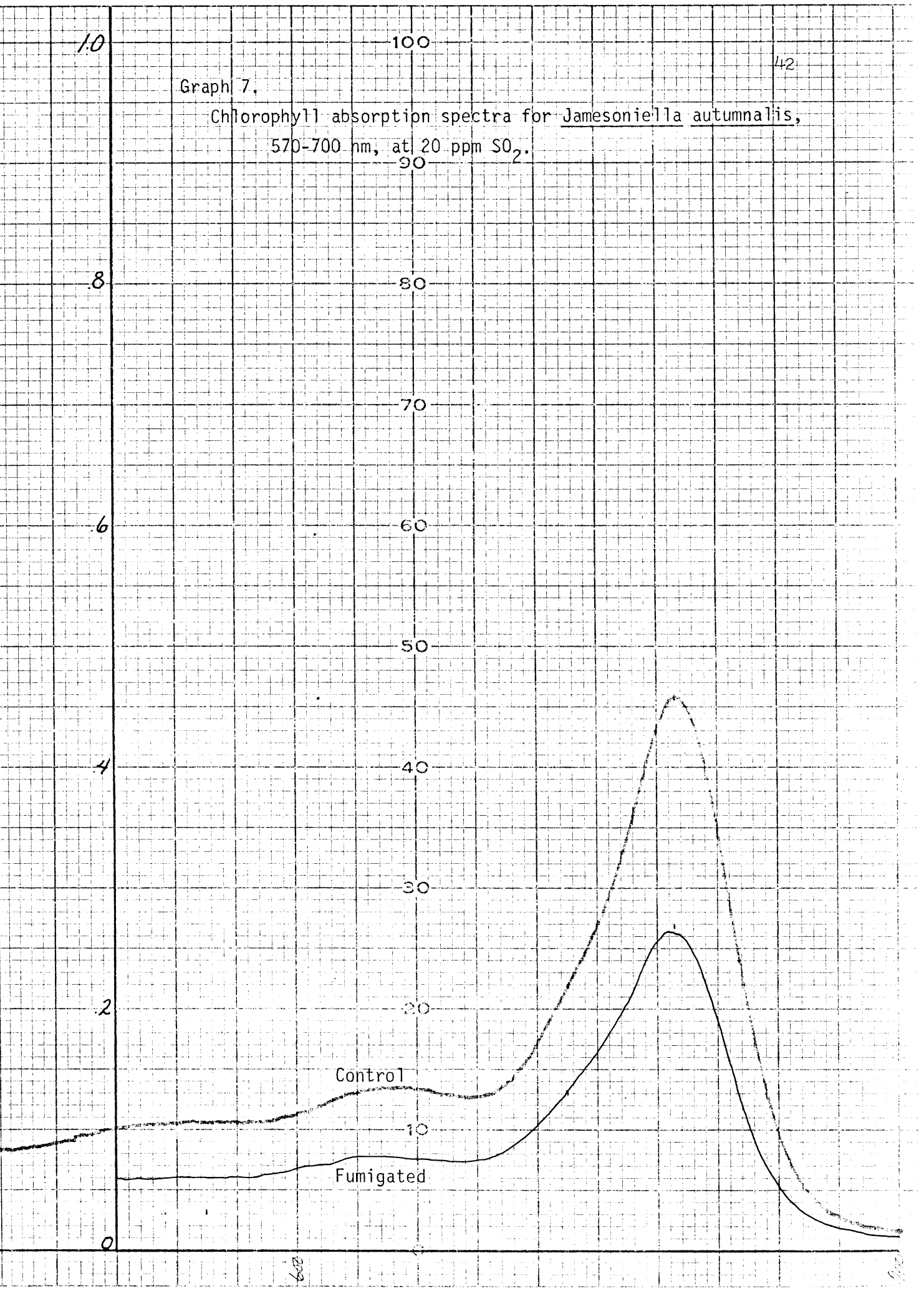
Graph 6.

Chlorophyll absorption spectra for Jamesoniella autumnalis, 570-700 nm, at 0.6 ppm $SO_2$.



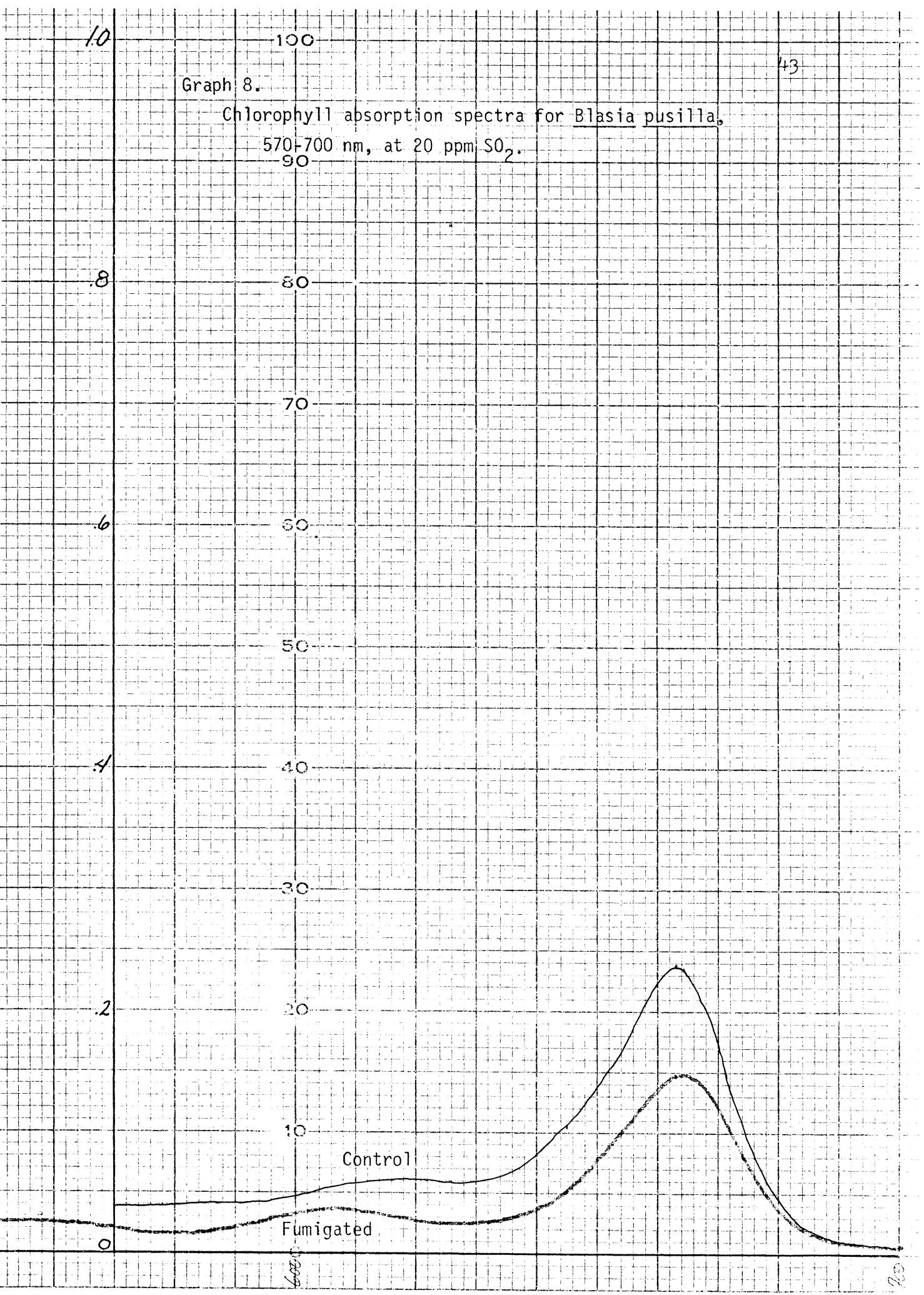
Graph 7.

Chlorophyll absorption spectra for Jamesoniella autumnalis, 570-700 nm, at 20 ppm $SO_2$.



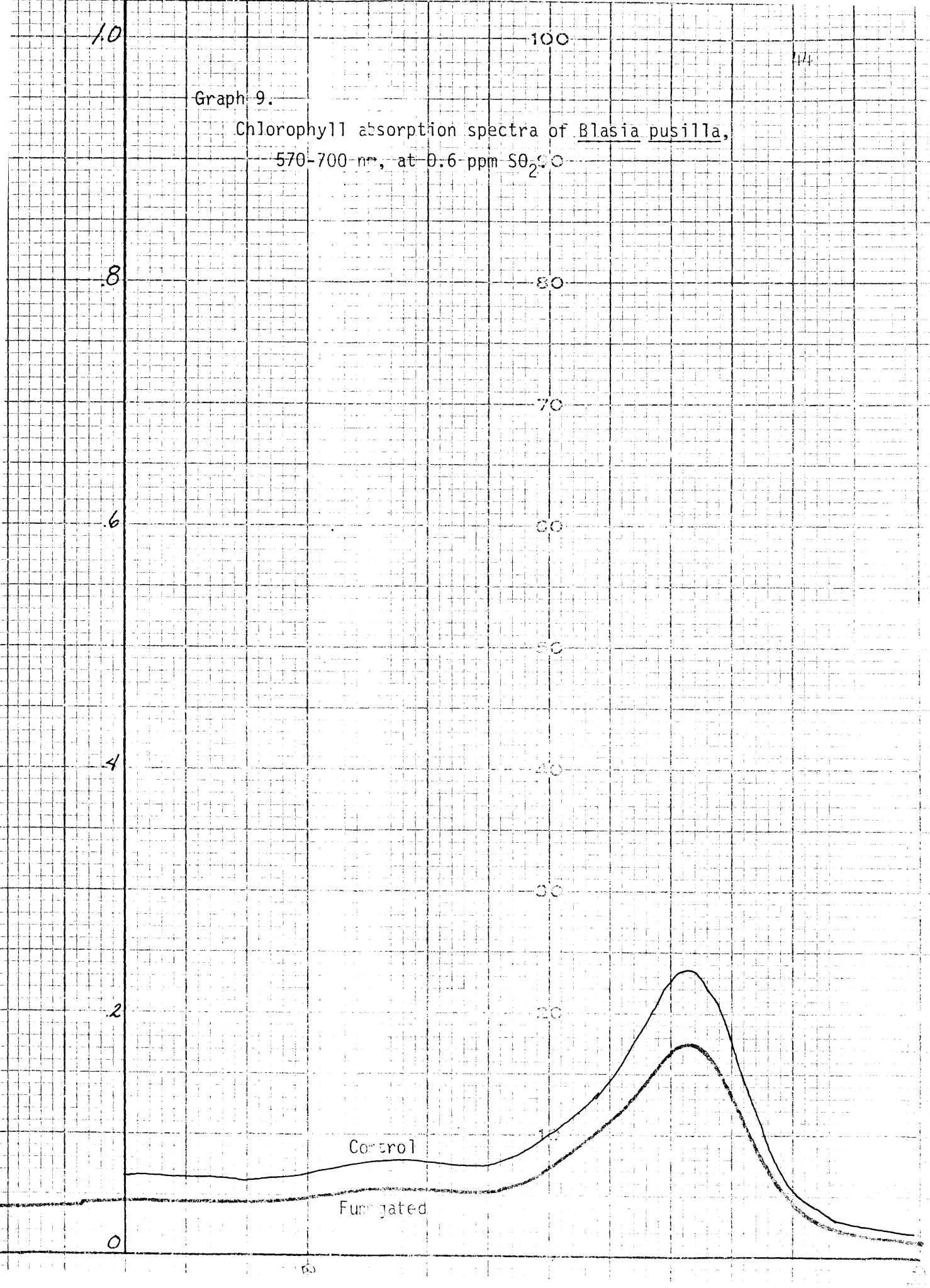
Graph 8.

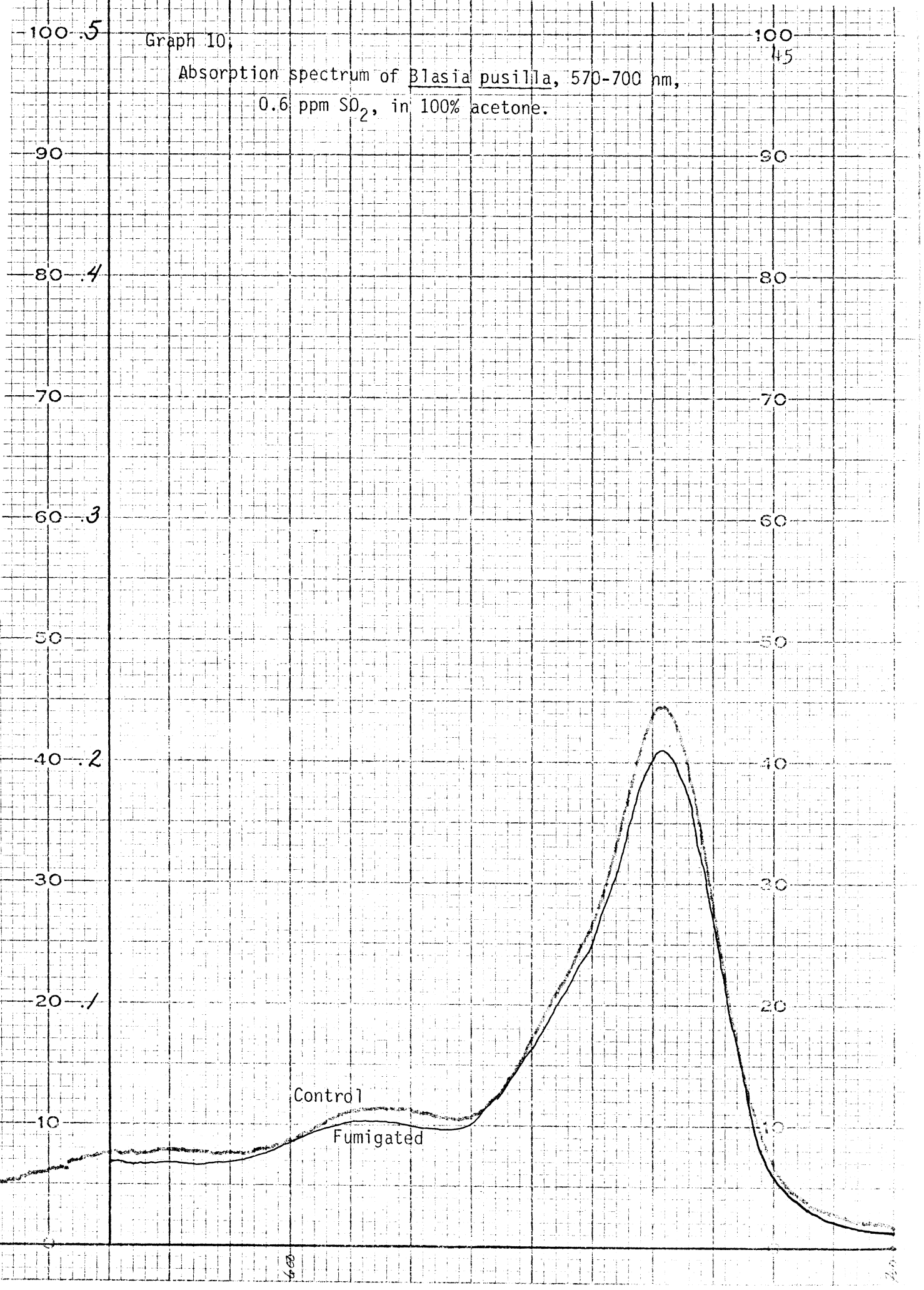
Chlorophyll absorption spectra for Blasia pusilla, 570-700 nm, at 20 ppm $SO_2$.



Graph 9.

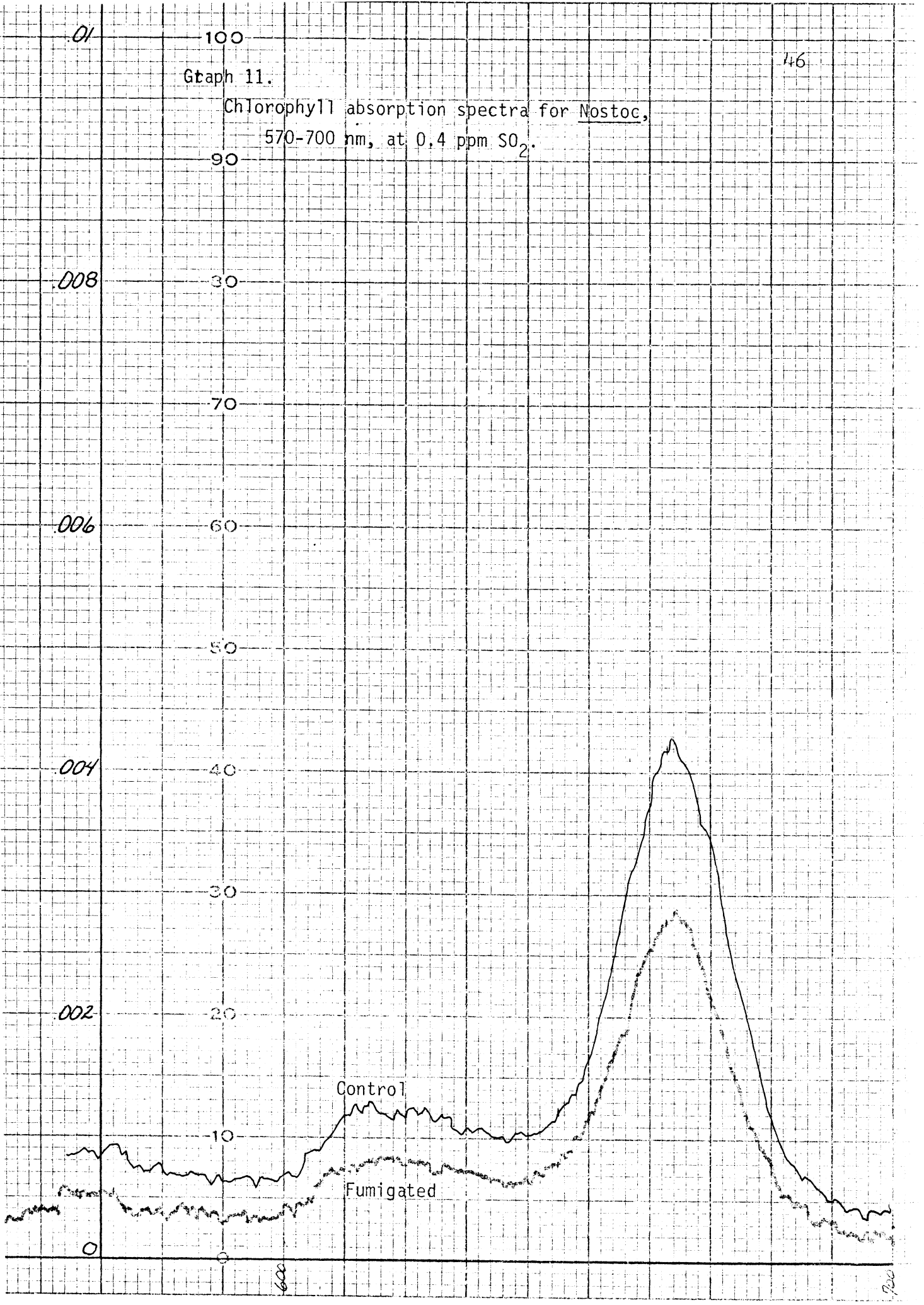
Chlorophyll absorption spectra of Blasia pusilla, 570-700 nm, at 0.6 ppm $SO_2$.




Graph 10.
Absorption spectrum of Blasia pusilla, 570-700 nm,
0.6 ppm $SO_2$, in 100% acetone.
100 .5
90
80 .4
70
60 .3
50
40 .2
30
20 .1
10
100
45
90
80
70
60
50
40
30
20
10
Control
Fumigated

Graph 11.

Chlorophyll absorption spectra for Nostoc, 570-700 nm, at 0.4 ppm $SO_2$.



## $SO_2$ Facts and Figures

A. Conversion of units:

1 ppm $SO_2$ = 2.86 $mg/m^3$ or 2858 $ug/m^3$

to convert ppm to $ug/m^3$ .... multiply by 2620

to convert $ug/m^3$ to ppm .... multiply by 0.38

B. U.S. Alert, Warning and Emergency Level Criteria

1. alert ..... 0.3 ppm, 24 hour average
2. warning ... 0.6 ppm, 24 hour average
3. emergency . 0.8 ppm, 24 hour average

Federal Register, Vol.36, No. 206, October 23, 1971, 15593

C. Air Quality Criteria

80-90 $ug/m^3$ ..... chronic vegetation injury, excessive leaf drop
140-160 $ug/m^3$ ... plant injury due to $O_3$ or $NO_2$ synergistic reaction
250 $ug/m^3$ ..... 50% reduction in visibility
800 $ug/m^3$ ..... injury to trees, shrubs

D. National Air Quality Standards in U.S.

| | Primary | Secondary |
|---|---|---|
| annual arithmetic mean ..... | 80 $ug/m^3$ | 60 $ug/m^3$ |
| 24 hour max. | 365 $ug/m^3$ | 260 $ug/m^3$ |
| 3 hour max. | - | 1300 $ug/m^3$ |

## Glossary of Terms

Acid rain - rain which contains products of oxidized sulphur or nitrogen, having a pH less than 5.6.

Acute injury - an injury, usually involving necrosis, which develops in a short time span (hours-days) due to a brief exposure to a high level pollutant.

Aerosol - a suspension of colloidal particles in a gas or mixture of gases, 0.01-100 u in diameter; measured in $mg/m^3$.

Air pollution - contamination of the atmosphere or alteration of the concentration of the existing components.

Biological indicator - plant species which are sufficiently sensitive to a specific pollutant to make them useful as indicators of the presence of that pollutant.

Chlorosis - a discoloration of plant tissue, yellowing or bronzing, due to a disruption of the chlorophyll.

Chronic injury - injury which develops after a long term or repeated exposure to an air pollutant, expressed as chlorosis, reduced growth, discoloration, etc.

Dusts - solid particles usually formed by a disintegration process; measured in $grains/ft^3$.

Fossil fuel - fuel derived from decayed organic matter from past geological ages, i.e., coal, oil, gas.

Fumigation - the natural or controlled exposure of plants to toxic gases.

Injury - any change in the appearance and/or function of a plant that is deleterious to the plant.

Mist - Liquid particles i.e., steam, fog.

Multiple source - sources of pollution in one area; residential, industrial.

Oxidation - the addition of $O_2$.

PAN - Peroxyacetyl nitrate; formed as a product of photochemical reactions involving nitrogen dioxide and hydrocarbons.

Particulates - finely divided particles of solid or liquid matter, i.e., dust, smoke, aerosols.

Photochemical smog - a combination of photochemical oxidants, smoke, fumes, and aerosols reacting with solar energy.

PPM - parts by weight or volume of pollutant per million parts by volume of air.

Primary pollutant - pollutants which are emitted directly from an identifiable source.

Reduction - the subtraction of $O_2$.

Secondary pollutants - pollutants produced in the air by reactions involving primary pollutants and/or other atmospheric constituents.

Sink - the places to which pollutants disappear from the air, i.e., soil, vegetation, water, structural bodies.

Smog - a mixture of smoke and fog.

Smoke - solid and/or liquid gas-borne particles, often less than 1 micron diam., formed by incomplete combustion of carbonaceous materials, visible.

Source - the place from which pollutants emanate.

Synergism - when the combined effect of two or more independent treatments is greater than the sum of each treatment alone.

Threshold - the minimum level of a pollutant or element necessary to induce plant injury or symptoms.

## Literature Cited


Arndt, U. 1971. Konzentrationsanderungen bei Blattfarbstoffen unter dem Einfluss von Luftverunseinigungen. Ein Diskussionsbeitrog zur Pigmentanalyse. Environ. Pollut. 2:37.

Arnold, F. 1892. Zur Lichenenflora von Miinchev. Ber. bayer. bot. Ges. Z. 1-76.

Bailey, J.L. and R.D. Cole. 1959. Studies on the reaction of sulphite with proteins. J. Biol. Chem. 234:1733.

Barkman, J.J. 1968. Air pollution: Proceedings of the first European Congress on the influence of air pollution on plants and animals. Wazeningan.

Brosset, C. 1973. Air-borne acid. Ambio. 1 & 2:1-8.

Cecil, R. and R.G. Wake. 1962. The reactions of inter- and intra-chain disulphide bonds in protein with sulphite. Biochem. J. 82:401.

Coker, P.D. 1968. The effects of $SO_2$ pollution on bark epiphytes. Trans. Sr. Bryol. Soc. 5:341.

de Cormis, L. Some aspects of the absorption of sulfur by plants subjected to an atmosphere containing $SO_2$. Air Poll. First Congress.

Dorries, W. 1932. ilber die Brauchfarkeit der spectroscopie Phaophytizerobe in der Rauchshaden - Diagnostik. Zeitschr. Pflanzenkr. Gallenk. 42:257-273.

DeSloover, J. and F. LeBlanc. 1968. Mapping of atmospheric pollution on the basis of lichen sensitivity. In: Proceedings of the Symposium on Recent Advances in Tropical Ecology (Ed. by R. Misra and B. Gopal). Intnl. Soc. for Trop. Ecol., Varanasi, India. p. 42.

Ferguson, P., and A. Lee, and N.B. Bell. 1978. Effects of sulphur pollutants on the growth of sphagnum species. Environ. Pollut. 16:151-162.

Ferry, B.W., M.S. Baddeley, and D.L. Hawksworth (editors). 1973. Air Pollution and Lichens. Toronto.

Frye, T.C. and L. Clark. 1937. Hepaticae of North America. Univ. of Washington, Seattle.

Geiger, R. 1965. The climate near the ground. Cambridge. Harvard Univ. Press.

Gilbert, O.L. 1968. Bryophytes as indicators of air pollution in the Tyne Valley. New Phytol. 67:15-30.

Haselhoff, E. and G. Lindau. 1903. "Die Beschadigung der Vegetation durch Rauch". Gebruder Borntraeger, Berlin.

Hawksworth, D.L. and F. Rose. 1970. Qualitative scale for estimating sulphur dioxide air pollution in England and Wales using epiphytic lichens. Nature. 227:145-148.

Hayatsu, H. and R.C. Miller. 1972. The cleavage of DNA by the oxygen-dependent reaction of bisulfite. Biochem. Biophys. Res. Commun. 46:120.

Hill, D.J. 1971. Experimental study of the effect of sulphite on lichens with reference to atmospheric pollution. New Phytol. 70:831-836.

Hocking, D. and M.B. Hocking. 1977. Equilibrium solubility of trace atmospheric sulphur dioxide in water and its bearing on air pollution injury to plants. Environ. Pollut. 13:57-64.

Hodges, Laurent. 1973. Environmental Pollution. Holt Rinehart and Winston Inc.

Howe, M.A. 1899. The hepaticae and anthocerotes of California. N.Y.

Howel, S.H. and E.N. Moudrianakis. 1967. Function of quantasome in photosynthesis. Proc. Natl. Acad. Sci. U.S.A. 58:1261.

Johnsen, I. and U. Sochting. 1976. Distribution of cryptogamic epiphytes in a Danish city in relation to air pollution and bark properties. The Bryologist. 79:86-92.

Kellogg, W.W., R.D. Cadle, E.R. Allen, Al Lazrus and E.A. Martell. 1972. The sulfur cycle. Science. 175:587.

LeBlanc, F. 1967. Epiphytes and air pollution. Air Pollution. First Congress.

LeBlanç, F. and D.N. Rao. 1966. Reaction de guelgues lichens et mousses epiphytigues a lanhydride sulfurenx dans la region de Sudburg, Ontario. Bryologist. 69:38-346.

LeBlanc, F. and D.N. Rao. 1973. Evaluation of the pollution and drought hypothesis in relation to lichens and bryophytes in urban environments. Bryologist. 76:1-19.

LeBlanc, F. and D.N. Rao. 1973. Effects of sulfur dioxide on lichen and moss transplants. Ecol. 54:612-617.

Likens, G.E. 1976. Acid precipitation. Chem. & Enzin. News. Vol. 54 (48):29-44.

Linzon, S.N. 1969. Symptomatology of sulphur dioxide injury on vegetation. Handbook of Effects Assessment, Vegetation Damage. Penn St. Univ.

Malhotra, S.S. and D. Hocking. 1976. Biochemical and cytological effects of sulphur dioxide on plant metabolism. New Phytol. 76:227-237.

Nash III, T. 1973. Sensitivity of lichens to sulfur dioxide. The Bryologist. 76:333-339.

Nash III, T. and E.H. Nash. 1974. Sensitivity of Mosses to sulfur dioxide. Oecologia (Berl.). 17:257-263.

Newberry, G. 1974. The influence of a sulfate-process paper mill on corticolons lichens. Bryologist. 77:561-576.

Noack, K., O. Wehner, and H. Griessmeyer. 1929. Untersuchunzen uber die rauchgasschaden der vegetation. Z. Angew. Chem. 42:123.

Nylander, W. 1866. Les lichens du Jardin du Luxembourg. Bull. Soc. Bot. France. 13:364-372.

O'Hare, G.P. and P. Williams. 1975. Some effects of sulphur dioxide flow on lichens. Lichenologist. 7:116-120.

Osmond, C.G. and P.N. Avadhani. 1970. Inhibition of the B-carboxyation pathway of $CO_2$ fixation by bisulfite compounds. Plant Physiol. Lancaster. 45:228.

Pearson, L. and E. Skye. 1965. Air pollution affects pattern of photosynthesis in Parmelia sulcata, a corticolons lichen. Science. 148:1600-1602.

Puckett, K.J., E. Nieboer, W.P. Flora, and D.H.S. Richardson. 1973. Sulphur dioxide: Its effect on photosynthetic $^{14}C$ fixation in lichens and suggested mechanisms of phytotoxicity. New Phytol. 72:141-154.

Puckett, K.J., E. Nieboer, W.P. Flora, and D.H.S. Richardson. 1974. Photosynthetic $^{14}C$ fixation by the lichen, Umbilicaria muhlenbergii, following short exposures to aqueous sulphur dioxide. New Phytol. 73:1183-1192.

Rao, D.N. and Brother F. LeBlanc. 1966. Effects of sulfur dioxide on the lichen alga, with special reference to chlorophyll. The Bryologist. 69:69-75.

Rao, D.N. and F. LeBlanc. 1967. Influence of an iron-sintering plant on corticolons epiphytes in Wawa, Ontario. Bryologist. 70:69-75.

Robinson, G.D. 1970. Long-term effects of air pollution. a survey. U.S. Environm. Protection Agency, Office of Techn. Inf. & Publ. Air Pollut. Techn. Inf. Center. Research Park Triangle. N. Carolina.

Shapiro, R. and B. Braveman. 1972. Modification of polyuridylic acid by bisulfite: effect on double helix formation and coding properties. Biochem. Biophys. Res. Commun. 47:544.

Sheridan, R., C. Sandersoa, R. Kerr. 1976. Effects of pulp mill emissions on lichens in the Missoula Valley, Mont. Bryol. 79:248-252.

Showman, R.E. 1972. Residual effects of sulfur dioxide on the net photosynthetic and respiratory rates of lichen thalli and cultured lichen symbionts. The Bryologist. 75:335-341.

Skye, E. 1968. Lichens and air pollution. Acta Phytogeogr. Suecica. 52:1-123.

Sundstrom, K. and J. Hallgren. 1973. Using lichens as physiological indicators of sulfurous pollutants. Ambio. 2 (1 & 2):15-20.

Syratt, W.J. and P.J. Wanstall. 1963. The effect of $SO_2$ on epiphytic bryophytes. In: Air Pollution: Proceedings of the first European Congress on the influence of air pollution on plants and animals. Wazeningan. 1968.

Taoda, H. 1973. Effect of air pollution on bryophytes: I. $SO_2$ tolerance of bryophytes. Hikobia. 6 (3/4):238-250.

Thomas, M.D., R.H. Hendricks and G.R. Hill. 1950. Sulfur metabolism of plants. Effects of $SO_2$ on vegetation. Ind. Eng. Chem. 42:2231.

Treshow, M. 1970. Environment and plant response. McGraw-Hill, N.Y.

Turk, R. and V. Wirth. 1975. Uker die $SO_2$ - Empfindlichkeit einiger Moose. Bryologist. 78:187-193.

Voth, P.D. 1941. Bot. Goz. Vol. 103, #2.

Wellburn, A.R., O. Majernik, F.A.M. Wellburn. 1972. Effects of $SO_2$ and $NO_2$ polluted air upon the ultrastructure of chloroplast. Environ. Pollut. 3:37.

Ziegler, I. 1972. The effect of $SO_3^=$ on the activity of ribulose-1-5-diphosphate carboxylase in isolated spinach chloroplasts. Planta. 103:155.